BIOPHYSICS

VOLUME 2, 1957

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1957

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PERGAMON PRESS

NEW YORK · LONDON · PARIS · LOS ANGELES

BIOPHYSICS

Biophysics is being published in translation by the Pergamon Press for the Pergamon Institute, a non-profit-making foundation for the furtherance and dissemination of scientific knowledge on the initiative and with the financial support of the National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

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ON THE POSSIBILITY OF ENERGY TRANSFER IN PROTEIN MOLECULES

Iu. A. Vladimirov and S. V. Konev

(*Received* 1 *August* 1956)

Different authors do not at present use the term "energy transfer" in the same sense. Hence we devote the first part of this article to defining the concept of energy transfer which we shall subsequently adhere to. Energy transfer is the radiationless transmission of an energy quantum from its site of absorption to the site of its utilization in the molecule or system of molecules, the distances involved being considerably greater than interatomic, without conversion to thermal energy of vibration, and without the donor and acceptor, between which the energy is transferred, coming into kinetic collision.

The question of energy transfer in living systems has a somewhat unusual history. While a theory is usually derived from the accumulated data of a science, the migration hypothesis was introduced into biology almost immediately after this phenomenon had been discovered in physics. In 1941 Szent-Györgyi [1] enumerated a number of biological problems in which it was necessary the solution of which, in his opinion, necessitated a resort to the concept of energy transfer. These were: Emerson and Arnold's theory of photosynthetic units, Kubowitz and Haas's data on the inactivation of urease, and Lacki's on the inactivation of fumarase; experiments on the action of crocin on algae, data from experiments on myosin, where it was considered that the number of molecules capable of reacting with ATP was much less than the number of molecules of myosin contracting; and finally, the considerations regarding functioning of respiratory enzymes. In 1942 [2], and again in 1946 [3], Szent-Györgyi brought forward some fresh evidence for energy transfer in protein systems: in 1946–47 the work of Bücher and Kaspers [4, 5] on the photo-dissociation of carboxymyoglobin appeared: in 1954 that of Bannister on the fluorescence of phycocyanin [6]; and in 1955 that of Ril [7] on the electrical conductivity of gelatin. A large number of papers dealing with the theoretical aspects and possible energy transfer mechanisms in protein systems have been published since 1938, and reviews relating to this topic have also appeared [8–13]. Many of the facts and views enumerated have by now become irrelevant. Thus the experiments on the action of crocin on algae, in which a change in the whole alga due to a few molecules of crocin (or even to one) was demonstrated, indicate only the spread of local damage, and do not indicate how it occurs. The same applies to experiments involving the action of ionizing radiations on living cells.

Szent-Györgyi's calculations, which showed that the number of myosin molecules reacting with ATP was considerably less than that participating in contraction, require confirmation. It is also impossible to consider the mechanism of electron transfer in cytochrome systems, as having been explained. Szent-Györgyi considers that, since

the cytochromes are attached to cellular structures, their haem groups cannot come into direct contact, and that electron transfer occurs via the proteins [2, 3]. However, Chance, on the basis of his spectrophotometric data [14], supposed that the cytochromes react by collisions, brought about by thermal vibration about the point of attachment to the stroma. Lipman supposed that the transfer of an electron from one cytochrome to another could occur through a soluble intermediate factor although Slater describes this factor as participating in the formation of energy-rich bonds only (stated according to [15]), and not in oxidation-reduction. In any case, this question has not been finally resolved, and Lipman's considerations are insufficient to demonstrate the migration of energy. Finally, the theory of photosynthetic units is apparently not related to the question under discussion, since we are here speaking of energy transfer between chlorophyll molecules, without the participation of protein [9].

Two aspects of energy migration in proteins must be distinguished. Firstly, can the energy be transferred from one site to another within the bounds of one protein molecule, i.e. does the protein molecule act as a whole from the point of view of energy, or are its various parts energetically distinct? For the radiologist or photochemist, when the changes in proteins and viruses due to an energy quantum, trapped in one part of the molecule, are being investigated, as also for the physical chemist investigating protein denaturation, and for the enzymologist concerned with the functional mechanisms of enzymes, for all these this is the side of the question that is probably of the greatest interest. On the other hand, the possibility of energy transfer throughout a system of protein molecules is of general biological importance. It is known that energy can be transferred between parts of a cell by diffusion of molecules with energy-rich bonds, i.e. the so-called "water" route. Whether energy can be transferred through the structure itself "in the dry way" is another question. "Structure" here is understood to mean, not morphological formations, but rather an organized colloidal-chemical system. This hypothesis is the more attractive, since upon migration the energy may be transferred to considerable distances almost without loss, and is also transferred to the point where it may most readily be utilized (the so-called "tropism" principle). But though hypotheses and suggestions such as these are attractive, it should be remembered that they often have no factual basis. The few signs so far obtained of energy migration in protein almost all imply migration within the bounds of one protein molecule. However, the importance of the problem demands a detailed and critical consideration of each instance.

I. INACTIVATION OF ENZYMES BY ULTRA-VIOLET LIGHT

In ordinary photochemical reactions the light acts at the site of absorption. Hence only radiations which are absorbed by the active group of an enzyme can destroy it. If light absorbed by other parts of the protein macromolecules can also have this destructive action, we may consider the energy as being transferred from these parts to the active group. Outwardly, this will manifest itself by spectrum of the action of light on the enzyme coinciding with the absorption spectrum of the whole protein molecule (and not with that of the active group).

Quantitative study of enzyme inactivation processes is accompanied by considerable difficulties, which are connected both with the complexity involved in calculating

quantitatively the number of light quanta absorbed during the whole time of the experiment by non-inactivated molecules, the proportion of which diminishes steadily, and with the difficulty of calculating the numbers of molecules inactivated. Warburg [16] obtained a mathematical solution to the problem in 1928: he developed a theory for determining the quantum efficiency for photo-dissociation of light-sensitive compounds. In the particular case where the process of enzyme inactivation was irreversible, and the molar extinction coefficients of active and inactivated enzyme were equal, the quantum efficiency can be calculated by the equation

$$\varphi = \frac{c_0 \ln (c_0/c)}{[1-\exp(-\beta c_0 d)]it}, \qquad (1)$$

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where c_0 and c are the initial and final molecular concentrations of active enzyme, of extinction β, respectively; i is the initial intensity of the incident light in moles of quanta; d is the thickness of the solution, and t is the time of irradiation. More recently McLaren has derived the same equation by a somewhat different method [17]. The experimental difficulties involved in producing line sources of ultra-violet which are of sufficient intensity and high purity, and the difficulty in calculating the number of quanta absorbed, were also overcome in Warburg's work on the quantum efficiency of the photo-dissociation of CO-iron compounds. For this purpose Warburg confined himself to one of the two possible limiting conditions of experiment: he used either a thick layer of optically dense solution, in which practically all the light was absorbed, and by careful stirring of the solution ensured that the absorption due to each component was proportional to its contribution to the total optical density; or, conversely, a thin layer of highly dilute solution, in which the light causing the dissociation was only very weakly absorbed (less than 10–15 per cent, or in some cases less than 1 per cent), and the absorption due to each component was proportional to its optical density, being independent of the absorption of other substances. Warburg used the first arrangement to determine φ manometrically (from the gaseous CO liberated), and used the second to determine φ by optical methods (from the change in optical density of the solution at a definite wavelength, as a measure of the CO removed from combination with the ferrous iron).

Warburg considered the mathematical treatment and experimental methods as correct, because the quantum efficiencies of the photo-dissociation of different complexes of CO with ferrous compounds were identical (being equal to unity), some of them (iron pentacarbonyl, CO-ferrous cysteinate, CO-pyridine-haemochromogen) being measured manometrically [16, 18], and others (CO-myoglobin) optically [5]. In 1929 Warburg, Negelein and Christian determined the quantum efficiency in the photo-dissociation of CO-haemoglobin [19], and his result was confirmed by Bücher and Negelein in 1942 [20]. This renders Warburg's calculations particularly conclusive.

Finally, the last, but not the least important, difficulty in the investigation of the quantum efficiency enzyme photo-inactivation, lies in calculating the number of inactivated molecules. This number is frequently not proportional to the rate of the enzyme reaction, and so it is necessary to construct a calibration curve [17], which, unfortunately, not all workers do.

Conditions which approximate most closely to the ideal are found in the work of Kubowitz and Haas [21] on the inactivation of urease, in which very dilute solutions of the enzyme were used, and the reaction rate was directly proportional to the concentration of active enzyme. In this case equation (1) above takes the simple form

$$\varphi = \frac{1}{\beta} c_0 \ln \frac{c_0}{c} \cdot \frac{1}{it}, \tag{2}$$

where the symbols have the same meanings as before.

These workers used a quartz monochromator to isolate the ultra-violet lines. Crystalline urease, carefully purified from traces of heavy metals, was inactivated in the same quartz Warburg vessels as were later used to determine the rate of decomposition of urea (manometrically, from the CO_2 evolved). The action spectrum was shown to coincide with the absorption spectrum; the quantum efficiency was constant for the wavelengths 196, 207, 227, 283, and 366 mμ. Thus short-wave ultra-violet quanta, which are absorbed by any amino acid, are just as effective as quanta which are absorbed only by the aromatic amino acids (283 mμ). Hence, Szent-Györgyi drew the conclusion that the energy migrated from any amino acid residue to the active group of the enzyme. However, the work of Landen [22] failed to confirm Kubowitz and Haas's results. He found that the quantum efficiency of urease inactivation was different at different wavelengths (Fig. 1). Landen used solutions which were 80 times more concentrated, and the irradiating beam passed through a depth of solution which was 10 times greater than that used by Kubowitz and Haas. The cuvette was narrow and long ($4 \times 10{\cdot}9 \times 40$ mm), which made the careful mixing, which is so necessary in this case, rather difficult, as well as the calculation of the number of quanta absorbed. The enzyme activity was determined from the ammonia liberated, but unlike Kubowitz and Haas, Landen constructed no calibration curves (as initial data for the calculation of the quantum yields). The formula proposed for the calculation contains an error, although, according to McLaren [17], Landen performed the calculation with the correct formula. Since Landen's experiments were

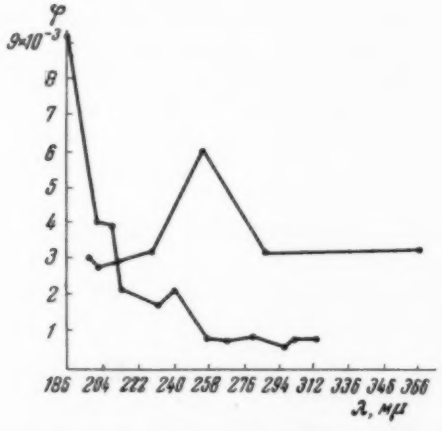

FIG. 1. Quantum efficiency of urease inactivation for ultra-violet light of different wavelengths, from Kubowitz and Haas [21] and Landen [22].

conducted under conditions completely different from those of Kubowitz and Haas, it is impossible to explain the divergencies in their results, the more so since Landen himself offers no explanation.

Other data in the literature on the quantum efficiency of enzyme inactivation are still less reliable, since they are either incomplete, or else the spectral distribution of quantum efficiency was not studied [17, 23–27]. The oft-quoted results of Gates [28] on the inactivation spectrum of pepsin gives different quantum efficiencies at the hands of the author and of Landen—which causes us to accept Gates' main conclusion, that the quantum efficiency of inactivation is constant at different wavelengths, only with reservations. The absence of a clear-cut picture as to the variation of quantum efficiency with wavelength in enzyme inactivation is probably explained not only by the methodological and mathematical difficulties, which have to a certain extent been overcome, but also by the basic incorrectness of the assumptions made in the experiments. At the root of all the calculations lies the hypothesis that all the molecules are equally liable to inactivation, no account being taken either of possible partial inactivation of enzyme molecules, or of possible summation of the actions due to many quanta within one molecule. However, even if this difficulty could be overcome, the fact that the quantum efficiency of enzyme inactivation was independent of wavelength would not serve to prove that the energy responsible for inactivation migrates. It is not necessary to destroy the prosthetic group to inactive an enzyme: it is sufficient merely to denature the protein. Photo-denaturation of proteins is well known and has a quantum efficiency of the same order as in enzyme inactivation (10^{-3}) [29].

The interpretation of Svedberg and Brohult's [30] experiments on the photo-dissociation of haemocyanin is no better defined (Ril explains these results by energy transfer). These workers showed that ultra-violet light of different wavelengths splits the haemocyanin molecule into two equal parts [30]. In this work it was not shown whether the parts were always exactly the same, and if so, what was the energy of the bond between them. This energy may possibly be very small, and the simple "heating" of the molecule by the quantum may be sufficient to dissociate it. (Haemoglobin can be split into two equal parts merely by changing the salt concentration in the medium in which it is dissolved.) Finally, since these workers did not measure the quantum efficiency, the interpretation of these experiments is rendered even more difficult. Thus photolyses of proteins and photo-inactivation of enzymes cannot be used to demonstrate energy transport in these instances.

2. EXPERIMENTS ON PHOTO-DISSOCIATION OF CO COMPOUNDS

Experiments on splitting off the carbon monoxide from CO-haemin-proteids do not differ in conception from those on the photo-inactivation of enzymes. The sole difference is that in the first case the site where the energy quantum is utilized is known accurately. As in the inactivation of enzymes, the fact that the action spectrum coincides with the absorption spectrum of the whole protein molecule (not just with that of the haemin part) is a criterion for the energy migrating to the haem.

The fact that light absorbed by the protein part of the molecule may participate in splitting the CO from the haem was first discovered by Warburg. Warburg studied the action spectrum of a respiratory enzyme [31, 32]. The light splits up the

complex between CO and the respiratory enzyme, and respiration recommences in the yeast cell suspension.

Fig. 2 shows that there is a strong maximum at $\lambda = 283$ mμ, which corresponds to the aromatic amino acids. The maximum is weak or absent in non-protein haem

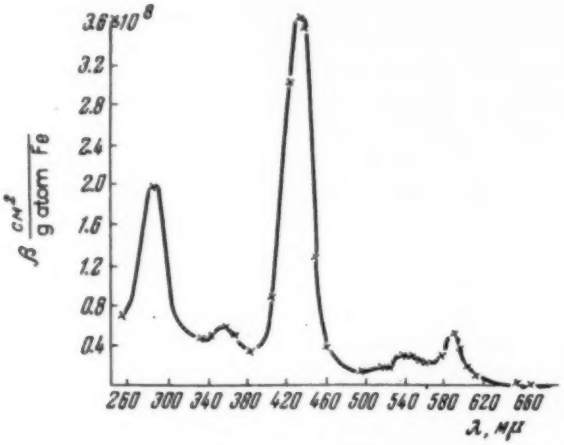

FIG. 2. Action spectrum of the compound of CO and respiratory enzyme (after Warburg [18]) β = absorption coefficient.

compounds, and is not so marked in haemoglobin or in myoglobin, although all haem compounds have otherwise very similar spectra [33]. Since the action spectrum of the respiratory enzyme has a pronounced protein maximum (283 mμ), in addition to the maxima typical of haem, we may conclude that light absorbed by the protein is as effective as that absorbed by the haem itself. No doubt arises as to the careful manner in which these experiments were carried out. Castor and Chance have recently confirmed these results for the visible spectrum [34], a completely different method being used: the light intensity required to produce a given rate of respiration was measured at different wavelengths, instead of the rate of respiration being measured: the rate at which the cell suspension used up oxygen was determined by polarography. Unfortunately, the spectral region which is of the greatest interest in this connection—the ultra-violet—was not studied in this way.

In spite of the very interesting nature of Warburg's results they are insufficient to resolve the problem under discussion, since the absorption spectrum of the respiratory enzyme is unknown, and it is only a probable hypothesis that the absorption band at 283 mμ is due to protein. Hence in 1946–47 Bücher and Kaspers [5] carried out some special experiments on the action spectrum of the reaction

$$\text{CO-myoglobin} + O_2 \underset{\text{darkness}}{\overset{\text{light}}{\rightleftharpoons}} \text{myoglobin-}O_2 + \text{CO}.$$

Optical methods were used to determine the quantum efficiency. The optical arrangements used are shown schematically in Fig. 3. The high-intensity light source was a high-pressure mercury lamp (2 kW), the lines being isolated with a purity not less than 95 per cent by filters. The essential condition—low optical density for the

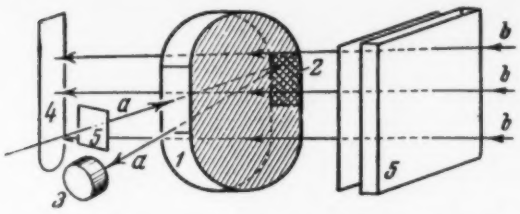

FIG. 3. Optical arrangement used by Bücher and Kaspers [5]:
(a) Standard beam; (b) Irradiating beam.
1—quartz cuvette (during the experiment in a thermostat); 2—mirror;
3—photocell; 4—bolometer; 5—filter combination.

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chemically active light, and, conversely, high density for measurement purposes—was ensured by using an elongated cuvette, by passing the light used for measurement through it twice, and by carrying out measurements at 405 mμ, at which wavelength the optical density of the CO-myoglobin complex is particularly large. The overall rate constant for the forward and reverse reactions together was calculated from the change of optical density with time of irradiation, while the rate constant for the reverse (dark) reaction was calculated from the change of optical density with time after stopping the irradiation. The difference of these quantities gives the photo-decomposition constant, which is directly proportional to the quantum efficiency of the reaction. The absolute quantum efficiency of the reaction was determined from the equation $\varphi = Z_i/i\beta$ where Z_i is the photo-dissociation constant, i is the intensity of the light passing through the solution, and β is the absorption coefficient. The quantum efficiency was found to be 1 at the five wavelengths at which measurements were made (280, 313, 334, 366, and 546 mμ), Fig. 4. The total absorption in the ultra-violet due to haemochromogen and the aromatic amino acids, taken in exactly the same amounts as in myoglobin, reproduces the myoglobin absorption. (At the same time the optical density due to the myoglobin haemochromogen is only about 60 per cent of the optical density at the protein maximum at 280 mμ. Hence Bücher and Kaspers concluded "that each light quantum absorbed by the tyrosine and tryptophane residues in the protein moiety has exactly the same effect in splitting a molecule of carbon monoxide from the iron in the haemin of myoglobin as does a quantum absorbed by the haem itself" ([5], p. 22).

Havemann and Wolf [35] do not agree with this conclusion. Starting from the observation that the absorption at 280 mμ in methaemoglobin varies with the binding of different substances to the haem, they consider Bücher and Kaspers' hypothesis, that all absorption at wavelengths below 300 mμ is due to the protein moiety as not being substantiated, and, moreover, that "in any case, it is impossible to say anything further about the transmission of energy through large distances" ([35], p. 260). The effects of different substances (CO in particular), combined with the haem, on the absorption at 280 mμ was of course known to Warburg and Bücher, since the relevant spectra were recorded in Warburg's laboratory: this effect is quite to be expected, since 60 per cent of the absorption at 280 mμ is due to the haem, and the possibility that the other 40 per cent is absorbed by the protein is not refuted. It appears that Bücher and Kaspers' "hypothesis" that below 300 mμ all absorption is

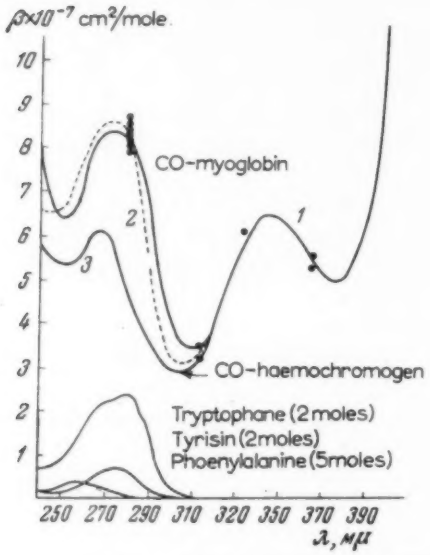

FIG. 4. 1—Photochemical action spectrum of CO-myoglobin;
2—Sum of the separate components (after Bücher [12]);
3—Absorption spectrum of CO-myoglobin and its components.

due to the protein, was actually never proposed by Bücher and Kaspers. It is thus difficult to agree with Havemann and Wolf's opinion that one cannot talk about an energy migration in this instance.

Another more serious mistake was made by Terenin and Krasnovskii [9] in interpreting Bücher and Wolf's experiment: they assumed the absorbed quantum may simply "heat up" the protein molecule, causing thermal dissociation of the CO-myoglobin complex. However, the energy required to perform this dissociation is fairly considerable—about 20–22 kcal [12, 36]—and it is difficult to believe that, if the energy of an ultra-violet quantum (100 kcal/mole) is distributed evenly over the degrees of freedom present in the whole protein molecule, the haem iron will receive one-quarter of the energy. Ril [7] states that an even distribution of this type would raise the molecular temperature by only 1°, and a temperature rise of this amount does not lead to the splitting off of the CO.

Finally, perhaps the most important objection is that the distances between the haem and the aromatic residues in myoglobin are unknown. Bücher considers their being in direct contact with the haem improbable, because the absorption spectra are additive; but this is scarcely conclusive, since he employs this same additivity as evidence of migration (see above). Evidence that the aromatic residues are not in direct contact with the haem must be sought elsewhere: firstly, it is known that in haemoglobin, and probably in myoglobin also, the haem is attached to the iminazol group of histidine, and to basic groups in the globin molecule [37], which latter probably belong to diamino acids: secondly, it is difficult to suppose that all nine aromatic amino acids in the globin are concentrated around the haem.

Similar objections were put forward by Broser and Lautsch [38]. They synthesized two compounds of porphyrin with the polypeptide (phenylalanine-glutamic acid), in which the molar ratios of porphyrin and amino acid were calculated to be 1: 100 and 1: 500 respectively [39, 40], and it was demonstrated that the quantum efficiency of the dissociation of CO from the iron of the porphyrin is equal to unity for the visible and ultra-violet regions. Since in this case all the aromatic amino acids absorb in the ultra-violet, while only one is in direct contact with the chromophore, then in our opinion, if Broser and Lautsch's data are confirmed, they will serve as a reliable indication of energy migration from the phenylalanine rings to the iron in the active groups.

3. THE FLUORESCENCE OF PHYCOCYANIN

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Although only less than a quarter of the energy in the absorbed quantum was used up in the chemical reaction with the CO-ferrous compounds, in Bannister's experiments [6] fluorescent quanta of considerable energy (about one-half of that initially absorbed) were emitted as fluorescence, and the possibility of thermal transfer of energy is completely excluded.

Bannister studied the relative fluorescent quantum efficiency from a phycocyanin solution, derived from a culture of the one-celled blue-green alga (Synechocystis), upon excitation by light of various wavelengths. The light from a low-pressure mercury lamp (250 W) passed through a quartz monochromator, and was focussed on a thin (1 mm) solution of phycocyanin. The energy flow in the exciting light was measured with a thermocouple, while the fluorescent intensity was measured with a photomultiplier.

Since the fraction of the exciting radiation absorbed by the solution was small (6 per cent), the number of quanta absorbed at any given wavelength is given by the product of the incident light intensity and the optical density of the solution.

The fluorescent intensity was measured in relative units (with respect to the intensity excited by irradiation at 405 mμ). The relative fluorescent quantum efficiency was found to be constant within 10 per cent limits for 11 wavelengths covering the range 254–405 mμ. Over this range the purity of the solution used was of extreme importance in this connection. The pigment was purified by repeated precipitation with ammonium sulphate or acetic acid, and the criterion of purity used was the ratio of extinctions at the absorption maxima of the chromophore (612 mμ) and of the protein (277 mμ). As the purity increased the ratio of the extinctions changed from 2·7 to 4·7, and the mean quantum efficiency for short-wave ultra-violet excitation changed from 0·6 to 0·9, while the mean quantum yield for excitation by long-wave ultra-violet remained approximately constant (Fig. 5). Consequently, a constant quantum efficiency in fluorescence is observed at different wavelengths, only if the purest phycocyanin preparations are used: as regards the ratio of extinctions for chromophore and protein (4·7), these were purer than the crystalline phycocyanin Svedberg used to determine the molecular weight (ratio 4·0). Bannister employed alkaline hydrolysis of the pigment (0·5 N NaOH at 100° for 45 min) to estimate the part due to protein in the total absorption of phycocyanin: the hydrolysate was separated into aqueous (amino acid) and ethereal (chromophore) fractions. When the pigment was pure 46 per cent of the absorption at 277 mμ (corrected for incomplete removal of the chromophore) was in the aqueous fraction (Fig. 6). So Bannister

concluded that light absorbed by the aromatic amino acids excited fluorescence just as effectively as that absorbed by the chromophore itself.

However, the importance of this to the problem of energy migration in proteins demands that we examine these experiments carefully (cf. [13]).

1. The gravest objection arises from the section of the work concerned with the estimation of the energy absorbed by the protein moiety of phycocyanin. Bannister

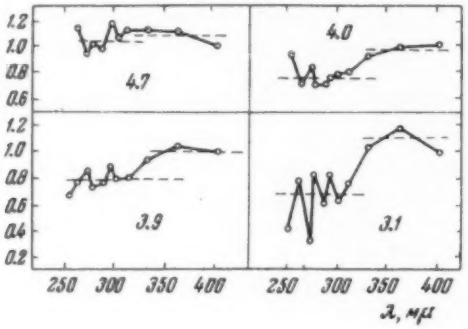


FIG. 5. Relative quantum efficiencies of the fluorescence of phycocyanin at different wavelengths. The numbers with the curves indicate the purity of the preparation (after Bannister [6], see text).

assumes that this fraction is identical both in the hydrolysate and in the native protein. But even if no account is taken of the fact that, when many dyes combine with proteins, their absorption spectra alter [41, 42], hydrolysis of this pigment causes a sharp change in the absorption spectrum of the solution, the principal maximum at 612 mμ disappearing, while the optical density at 275 mμ increases by a factor 1·5 (Fig. 7).

2. The absorption due to the chromophore itself cannot be calculated directly for the hydrolysate, since only part of it goes over into the ether fraction. The magnitude of this part can be calculated from the ratio of optical densities of the ether fraction and the whole acidified hydrolysate at 330–370 mμ, in which region amino acids do not absorb.

3. Bannister did not allow for the possibility that some of the amino acids go over into the ether fraction, along with the chromophore: but Lemberg [43] had already pointed out this possibility. However, this particular error will only reduce the absorption assigned to the amino acids.

4. Although Bannister states the pH of the solution when measuring the quantum efficiency [6, 3], no account of pH was apparently taken when measuring the absorptions of the components, since the pH values for the hydrolysate and the fractions are not given at any point in his paper: only the "alkaline" and "acidified alkaline" hydrolysate are referred to. Incidentally, the pH can affect both the distribution of the components between the fractions, and also their absorptions (e.g. the extinction of tyrosine can change with pH by a factor 1·7 [17]). It is fairly difficult to estimate the effect of all these inaccuracies on the correctness of Bannister's main conclusion. The most questionable step, as Terenin and Ermolaev have already pointed out [13],

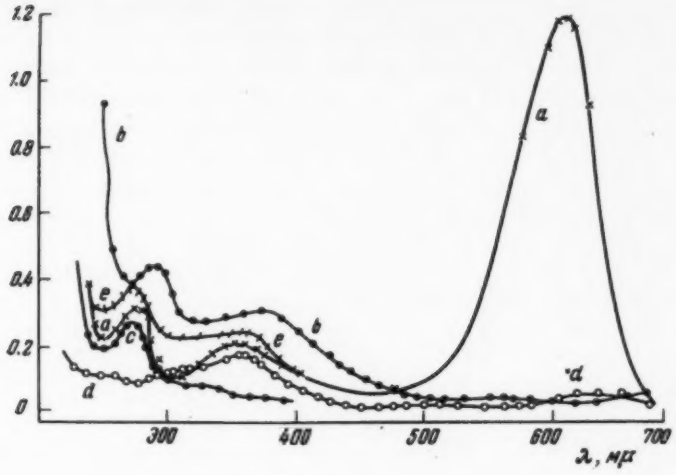


FIG. 6. Absorption spectra:

(a) Native phycocyanin; (b) alkaline hydrolysate; (c) water fraction of acidified alkaline hydrolysate; (d) ether fraction of acidified alkaline hydrolysate; (e) acidified alkaline hydrolysate. (After Bannister [6].)

is that of transferring the data from the hydrolysate to the native protein, since the spectrum changes on hydrolysis. The increase in extinction at 275 mμ observed on hydrolysis may be dependent on changes in the absorptions of both protein and chromophore, and therefore the absorption assigned to the protein must be altered. However, Bon [44] showed that the absorption of the proteins α—and β—crystallins and egg albumen are equal to the sum of the absorptions due to the amino acids (in the free condition) of which they are composed. Hence a more likely hypothesis is that the increase in phycocyanin absorption at 275 mμ after hydrolysis is due to the

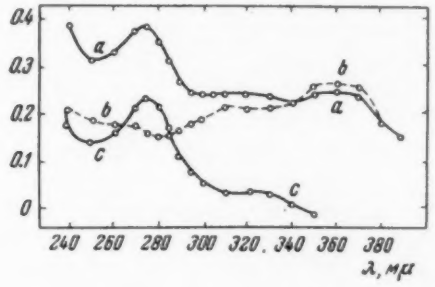


FIG. 7. Absorption spectra:

(a) Acidified alkaline hydrolysate; (b) Chromophore (corrected—see text); (c) Absorption due to the constituents of the protein. (c—a—b, from Bannister [6].)

chromophore, the more so as its spectrum changes markedly in the visible region. In this case the fraction of the absorption due to the protein will be, not 46 per cent, but 69 per cent of the total, which favours Bannister's main conclusion. The same applies to the possibility that part of the amino acids go over into the ether fraction. The absence of any change in the fluorescent spectrum has no bearing on the quantum efficiency, since Vavilov has shown that the fluorescent spectrum does not depend on the exciting wavelength, for monodisperse molecules in solution. Finally, the possibility that the pigment reabsorbs ultra-violet fluorescent radiation from the protein [13] can be neglected, on account of the small optical density of the phycocyanin solution. All these arguments compel us to consider the migration of energy in phycocyanin as being an authenticated fact.

4. POSSIBLE MECHANISMS OF ENERGY MIGRATION IN PROTEINS

In contemporary physics three main mechanisms are suggested by which energy can migrate in non-biological systems: electronic migration, the migration of excitons, and the resonance transfer of excitation energy between molecules (Vavilov-Perren [11]). Correspondingly, discussion of the mechanism of energy migration in proteins has revolved around these three possibilities [10].

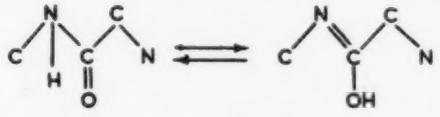
The hypothesis of conduction bands in proteins

Energy migration by electrons was discovered in luminescent inorganic crystals. When such crystals absorb a light quantum, an electron from some atom passes over into an energy-level which spreads throughout the whole crystal (the so-called conduction band), and may remain there until it encounters some lattice defect, such as an internal flaw or extraneous atom. This type of migration must be accompanied by the sensitized fluorescence of impurities, by phosphorescence and by photoconductivity, although the first is not peculiar to this type of migration of energy. Jordan had already pointed out that generalized energy-levels might exist in proteins in 1938 [45]. Szent-Györgyi reviewed this hypothesis in 1941 [1]. Starting from the straight line disposition of the amino acid residues in the polypeptide chain, he pointed out the analogy between proteins and metals (although it would be more accurate to compare proteins with semiconductors). In 1947 he performed experiments on dyed proteins in order to confirm his hypothesis.

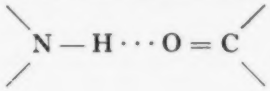
Some fluorescent dyes do not fluoresce as ordinary solutions in water, but acquire the power to do so when combined with certain proteins: Szent-Györgyi concluded from this that generalized electronic levels exist in proteins. But it is found that different proteins cannot call forth this phenomenon to the same degree. Seven per cent serum does not show the effect, while dyed actin and myosin fluoresce weakly: and only a gelatin gel gives a marked effect. When the fluorescence was marked, it was found that its intensity increased with time up to some constant value, the length of the latent period decreasing with the density of the gel. In the fluorescence of dyes in rigid media, which is known to depend on the molecule passing over to a metastable state [46], the phenomenon of fluorescence in the absence of oxygen (e.g.

in vacuo) is observed. Since Szent-Györgyi used photo-dynamically active dyes, which oxidize the protein and thereby consume the oxygen, phosphorescence of the absorbed dyes was observed in these experiments because oxygen was absent from the gel, and not because generalized electronic levels exist in the proteins [9].

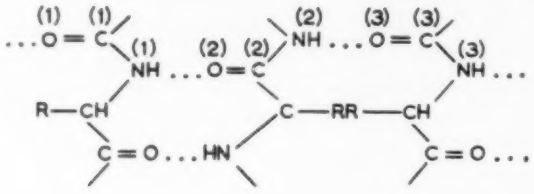
Photo-conductivity is the effect most characteristic of electron-migration. Szent-Györgyi made the first observations in this field in 1941. He studied the conductivity of vacuum-dried films of dyed gelatin in the dark and in light. The conductivities of the films increased by factors of 1·5–5 under illumination. The magnitude of the effect depended on the nature of the dye and on the experimental conditions (e.g. dyed casein which had been soaked in paraffin gave no photo-conductivity). But as Terenin and Krasnovskii [9] pointed out, this effect may be dependent on the dye alone, the more so since they showed that the effect was present with pure dye films, but was not found on irradiating dry proteins and crystalline amino acids with ultra-violet light. Ril's [7] recent measurements of the conductivity of pure gelatin, in which an increase in conductivity was observed on irradiating with ultra-violet light, also do not, in our view, indicate that there are conduction bands in proteins. In these experiments the conductivity of vacuum-dried gelatin was measured at a voltage gradient of 5000 V/cm. The conductivity was found to be 8×10^{-19} cm^{-1}, increasing by a factor 20 upon irradiation at 366 mμ, and by a factor 50 at 254 mμ. The photo-current did not change on cooling to −78°. However, it is quite probable that the extremely small absolute value of the photo-current observed (5×10^{-14}A) can be explained by the presence of traces of readily ionizing contaminants: this is all the more likely, since gelatin absorbs only very weakly in the 366 mμ region. Thus, neither the phosphorescence of dyed proteins, nor the photo-conductivity experiments, support the hypothesis of a conduction band in proteins, in the form originally proposed. Later, some variants of this hypothesis were proposed; these, as distinct from Szent-Györgyi's original views, which assumed in essence that any excited electron could migrate in any direction, were derived from hypotheses relating to the direction in which energy migrates in a system of conjugated double bonds. In such a system, as has been demonstrated for aromatic compounds and polyenes, the π-electrons can be transferred throughout the whole system, and so may become generally distributed. Schmidt [47] in 1947 suggested that a system of conjugated double bonds could form along the polypeptide chain by tautomeric rearrangement:



However, this hypothesis has never been confirmed experimentally. In 1949 Evans and Gergely [48] proposed a scheme for the migration of π-electrons across neighbouring polypeptide chains. Using X-ray structural data and quantum-mechanical calculations on the ψ function for the π-electrons in the system



they showed the possibility of a displacement of the π-electrons of the N atom and the C = O group. The proposed mechanism for migration of energy they explained as follows:



However, they proposed no factual basis for their hypothesis, if we neglect their calculations, which were, in any case, incomplete. These ideas only received an experimental test afterwards when in 1953 Eley *et al.* [49] studied the conductivities of dry proteins and compounds with conjugated double bonds: they found that the electrical conductivities of crystilline albumin, edestin, and fibrinogen followed the same exponential law as those of cyclic hydrocarbons, i.e.

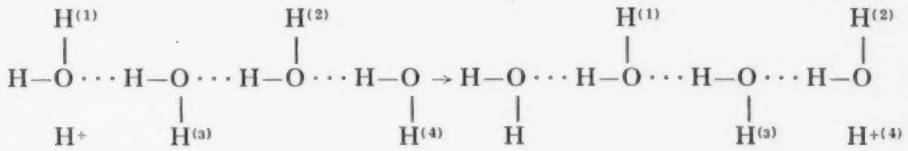
$$\chi = \chi_0 \cdot \exp(E/RT) \tag{3}$$

From the fact that the value of $2E$ found experimentally (48–60 kcal/mole) was close to the theoretical value given by Evans and Gergely (46 kcal/mole), they deduced the existence of conduction bands in proteins. Although care was taken to exclude the effect of oxygen and moisture, and the data are in good agreement with equation (3) for the temperature range $+70°$ to $+270°$, this still gives us no information as to the nature of the activation process, and a simple coincidence in activation energy is insufficient to establish identity of mechanism. In addition, the proteins used underwent such drastic treatment (complete desiccation, heating to 270°, and high pressure) that they were probably dealing with proteins that were extremely altered, and their data can scarcely be extended to the native proteins.

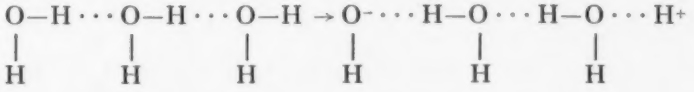
Thus even the most popular theory of energy transfer in proteins—the theory of electronic conductivity—has not been convincingly confirmed. Moreover, Evans and Gergely's theoretical scheme, which is the soundest, is in marked conflict with the absence of light absorption by proteins in the visible region. Actually if the excitation energy required to raise an electron in a protein to the conduction band is 46 kcal/mole, proteins should absorb strongly in the red region of the spectrum. In fact, proteins are colourless.

It is evident that similar arguments caused Ril to propose a proton mechanism for energy transfer in proteins, analogous to that he developed for similar transfers in ice [7]. From the temperature-dependence of conductivity in naphthalene crystals, Ril concluded that the conductivity is basically due to an incomplete thermal ionization. By applying a strong external electric field the reverse recombination of ion-pairs may be retarded. Such a long-lived ion-pair can itself stabilize the neighbouring thermally produced pair and a chain of ion-pairs is thus formed between the electrodes,

the current passing along this chain[1]. Ril also proposed an analogous explanation for the conductivity of ice. If an ion-pair OH^- and H_3O^+ forms at some point in the crystal lattice, the proton in the hydroxonium ion may go over to the neighbouring water molecule, as below:



The difference between this and the Grottus scheme for proton transfer in water:



consists of the formation of long-lived chains, by which many protons may be transferred; as in the case of water, after each proton transfer, molecular relaxation must occur.

Hence ice is considered as a proton semiconductor. The water adsorbed on a protein may, in Ril's opinion, also act as a proton semiconductor. While the stabilizing factor in ice is its crystal structure, in this case the polypeptide chain is the stabilizer, since it binds the water by means of hydrogen bonds. The experimental basis for this hypothesis is derived from the electrical conductivity of gelatin. While anhydrous gelatin is virtually an insulator, gelatin containing one molecule of water per amino acid residue is a conductor at a potential gradient of 6000–110,000 V/cm. Three main effects were observed:

1. The Pauli effect (increase in conductivity at high potential gradients).

2. Exponential variation of the conductivity with temperature over the range $-10°$ to $+20°$, in accordance with equation (3) above, with $E = 21$ kcal/mole (as in the case of ice).

3. E is independent of the potential gradient.

Since the data obtained with water and gelatin were comparable Ril supposed that the mechanisms were also comparable.*

The question whether proton transfer can cause energy migration implies the question as to how energy of ionization is transformed to energy of molecular vibration and rotation. This transformation certainly occurs in water: energy is expended in the relaxation. In ice and proteins the proton energy is lost neither by relaxation nor by overcoming potential barriers. Consequently energy of ionization migrates simultaneously with the proton.

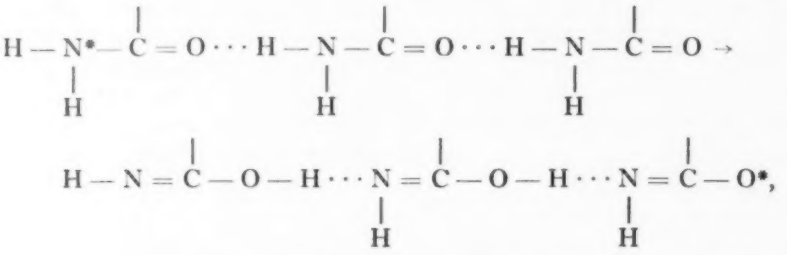
In spite of this hypothesis being entirely original, and even supposing it to be correct for ice, it can scarcely be considered as proven for proteins. All the pheno-

* Following Ril, a number of workers have proposed proton conduction as occurring in solid organic acids: this has been confirmed experimentally by the evolution of hydrogen.

mena enumerated above indicate only that the process involves an activation, but they give no clue as to its mechanism. As in the experiments of Eley *et al.*, the fact that two activation energies happen to coincide is quite insufficient to prove that their mechanisms are identical. Finally, the exponential variation of electrical conductivity with temperature is only observed at temperatures below $+20°$, i.e. outside the range which is of greatest interest in the biological field. It is evident from a comparison of Eley's and Ril's data that the electrical conductivity involves an activation, although, apparently, quite different processes are being activated. The advantage of Ril's hypothesis over that of electron-conduction is that no high energy of electron-excitation is required, and the absence of an absorption band in the visible region is explained.

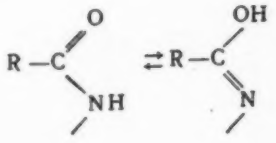
Energy migration by excitons

The exciton concept was introduced by the Soviet physicist Frenkel in 1932. As distinct from the migration of energy in a semiconductor, where the energy is carried by material particles, in this case the energy is transferred through the crystal lattice as energy of excitation, i.e. as an exciton. Frank and Livingstone [10] consider that such energy-transfer mechanisms occur in Wirrz's scheme for the protein molecule:



(The asterisk indicates the excited atom.)

where intramolecular electron-transfer is balanced by intermolecular proton transfer. In this case the transfer of excitation energy to considerable distances can only occur with the tautomeric states of equal energy values:



and if no energy is degraded to heat.

Some doubt may occur whether both conditions are found in proteins. Firstly, Wirrz himself states that the free energy of such a tautomerization is commensurate with 1 kcal/mole, i.e. is fairly considerable. Secondly, in order that the excitation energy should not be degraded to energy of thermal vibration, a high rate of exciton

transfer and high rigidity of the basic crystal structure are required. Rigidities of this order (which are comparable with those for the crystals of aromatic hydrocarbons) are extremely improbable in proteins. It is also scarcely possible to imagine that the transfer of a proton (the mass of which is comparable with the masses of the other atomic nuclei) would not cause molecular vibration. (This comment is, however, also applicable to Ril's hypothesis.) Finally, Wirrz did not put forward a single piece of factual evidence in support of his hypothesis. This all serves to make the hypothesis of energy transfer by excitons improbable.

Resonance transfer of energy

Unlike the types of energy migration discussed above, migration of energy between molecules by resonance (after Vavilov-Perren) does not require any definite organized crystal structure, but three basic conditions must be fulfilled if excitation energy is to be transferred.

1. The energy donor must be fluorescent.

2. The fluorescence spectrum of the energy donor must overlap the absorption spectrum of the acceptor.

3. The donor and acceptor of the energy must be sufficiently close together (not more than 100 mμ).

All these conditions are apparently fulfilled in protein molecules.

1. All amino acids fluoresce in the visible region when adsorbed on paper, although it is so far not clear whether they fluoresce in solution.

Weber pointed out [51] that the early work of Vles on the fluorescence of proteins in the blue region was invalidated because it was caused by impurities, since the radiations most strongly absorbed by the proteins were least effective in exciting fluorescence, and the fluorescent intensity fell off on purifying the protein. According to Bannister's calculations [6], aromatic amino acids, which absorb in the range 270–275 mμ, should fluoresce in the ultra-violet region (300-400 mμ), and not in the visible. The recent work of Shore and Pardee [52] shows that aromatic amino acids, both in solution, and combined in proteins, fluoresce in the ultra-violet below 350 mμ. Using a quartz monochromator, and filters to cut out the exciting light, and measuring the intensity of fluorescence with a photo-multiplier, they calculated the quantum efficiency in fluorescence for carefully purified preparations of crystalline proteins and amino acids. The quantum efficiency of these compounds (1–6 per cent) was on the average of a lower order of magnitude than usual.

2. Hence aromatic amino acids fluoresce in the near ultra-violet. On the other hand, haem and the chromophore of phycocyanin absorb fairly strongly in this region, which probably also explains the 100 per cent energy migration observed by Bannister and by Bücher and Kaspers. If the acceptor absorbs weakly in the region where the protein fluoresces, as was the case in the latest work of Shore and Pardee [54] on the sensitized fluorescence of proteins conjugated with dyes, the energy migrates with a quantum efficiency which is considerably less than unity. When the number of dye molecules and the number of aromatic residues to the protein molecule is increased, the energy transfer becomes more effective, which is quite to be expected, if account is taken of the increased spectral overlap in both cases. Conversely,

quenching of the protein fluorescence is accompanied by cessation of energy transfer.

3. Finally, the last condition—sufficient proximity of donor and acceptor—must be complied with for the majority of proteins (see table).

TABLE 1. DIMENSIONS OF PROTEIN MOLECULES
(after Gaurovitts [53])

Protein	Molecular dimensions Å	Source of data
Insulin		
Dry	144 × 83 × 34	X-ray analysis
Moist	130 × 74·8 × 30·9	
Lactoglobulin		
Dry	67·5 × 67·5 × 154	
Moist	60 × 63 × 110	
Ribonuclease		
Dry	30·8 × 38·5 × 53·5	
Moist	28·7 × 29·3 × 45·2	
Edestin	70 × 70 × 70	Electron-microscope
Myosin	(5-100 Å²)x	

x = some thousands.

The table shows that the separation between the most distant points of the protein is comparable with 100Å for the usual globular proteins, and the energy of any aromatic ring may be transferred to an acceptor located near the centre of the protein molecule. Thus all three conditions for resonance energy migration may be considered as fulfilled in proteins. On the other hand, this mechanism does not require any special additional structural features. For example, Shore and Pardee [54] found that denaturation of the protein did not impair the efficiency of energy transfer to the dye.

The spectral region most effective in exciting fluorescence was that around 280 mμ: this tells us that the amino acids and the protein are fluorescing in this case, and not impurities. Unfortunately, Shore and Pardee did not measure the fluorescent spectra, and they only state that the maximum radiation lies below 350 mμ. Using a mercury lamp type PRK-2 to excite the fluorescence, and an SF-4 spectrophotometer to measure it, we obtained the fluorescence spectra for a 1×10^{-3}M solution of tyrosine in neutral, weakly alkaline, and weakly acid solution, the maximum being around 315 mμ, which confirms Shore and Pardee's data.

CONCLUSIONS

As in any new problem, energy migration in proteins is at present in the stage where facts are being accumulated, and as yet we possess neither exhaustive proofs nor verified theories. We can only say that energy can migrate in proteins in some particular instances. As to whether energy transfer can occur in any protein, and whether a protein molecule is a self contained unit from the point of view of energy, or whether only some proteins act in this way, no answer is at present forthcoming. The theoretical considerations put forward by Shore and Pardee show that resonance transfer of energy is the most probable, but a final answer to this question lies in the future.

Translated by J. E. S. BRADLEY

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THE TECHNIQUE AND SOME RESULTS OF THE INVESTIGATION OF THE MECHANICAL MANIFESTATIONS OF HEART ACTION IN MAN IN THE NORMAL AND PATHOLOGICAL STATES

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Until recently the main attention of cardiologists was directed to the investigation of the electrical, and in part, to the auditory, manifestations of the heart's action, as recorded by the electrocardiograph and the phonocardiograph. The mechanical effects arising from the contraction of the heart and the circulation of the blood in the large vessels have not, with rare exceptions, been investigated in man mainly on account of the absence of convenient, sufficiently sensitive and exact means of recording them. In recent years the position in this field of physiology and pathology has changed and the mechanical manifestations of heart action have begun to be investigated by many cardiologists. In so far as the mechanical effects are a direct index of the contractile function of the heart they deserve, to that extent, serious attention, and should be submitted to detailed analysis in the normal and in pathological subjects. The dynamics of the separate phases of the cardiac cycle should, in our opinion, be examined in particular detail.

Cardiac systole is accompanied by a complex of various mechanical processes occurring in the body. They manifest themselves physiologically by changes of blood pressure in heart and blood vessels, the cardiac impulse, the pulse waves in the arterial wall, ballistic phenomena, i.e. a displacement of the body dependent on the reaction or "recoil" arising from the ejection of the blood from the heart into the main vessels, and its movement in the vascular system, the shifting of the heart mass and that of the blood, changes of position and form of the heart, and a number of other factors. For the registration of these numerous phenomena the following techniques are employed: recording of the blood pressure in the heart cavities and the vessels, cardiography, sphygmography, ballistic cardiography, dynamocardiography (cardiohaemodynamography), electrokymography, and others.

At the present time in the application of all these techniques electrical recording is utilized, based on the conversion of the mechanical magnitudes into electrical, and the recording of changes of these latter by means of electromagnetic and cathode-ray oscillographs. The greatest merits of these technical methods of recording lie in their great sensitivity, the high intrinsic vibration frequency of the instruments recording the process under observation (of the "pick ups"), i.e. their small inertia, and the possibility of recording at a distance. An advantage of these methods is also—and this is of great interest in cardiology—that they present a means of simultaneous investigation and comparison of the heterogeneous physical and physiological processes—for example, the electrical auditory and mechanical phenomena of heart

action. Such investigations give much fuller, many-sided and comprehensive results, and permit the elucidation of the relation between the various processes bound up with cardiac activity.

The practical application of electrical methods of recording heart action in physiological laboratories and in clinics makes it possible to study this action in the whole healthy or diseased human organism in a normal physiological condition, without causing him any deleterious excitement. Thus these methods of study satisfy the basal requirements of the methods of physiological research laid down by Pavlov.

In this article we give some data on the mechanical processes bound up with cardiac activity, which we have obtained over a period of recent years with the help of a technique worked out in collaboration with V. S. Gurfinkel, T. S. Vinogradova, E. L. Romel, and Ia. S. Iakobson. We at first called this technique cardio-haemodynamography [1], but as it makes possible a keen analysis of heart action we now call it dynamocardiography.

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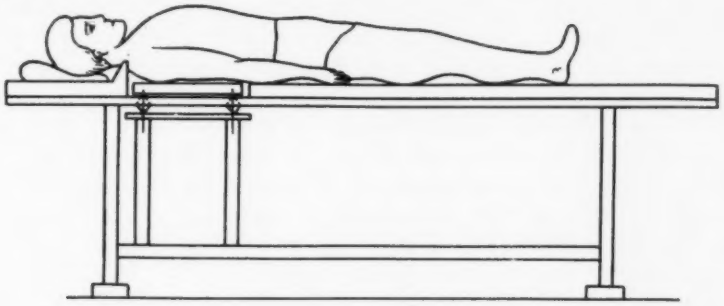

FIG. 1. Diagram of the dynamocardiographic table with recording apparatus.

In applying this dynamocardiography the human subject lies on a special couch or table with a recording arrangement exactly under the thorax (Fig. 1). This recording arrangement consists of two rigid metallic or plastic slabs between the corners of which there are elastic elements in the form of steel rings or cantilevers. To these elastic elements there are fixed wire strain gauges with ohmic resistance. These are connected between themselves so as to form a four-armed Wheatstone bridge. The wire strain gauges situated to the right and left of the foremost elastic elements form two opposite arms of the bridge, and the gauges situated to the right and left of the hinder elastic elements form the other two opposing arms of the bridge joined up to the first. Thus in the arrangement used all the arms of the bridge are active, i.e. the magnitude of their resistance is altered by the action of the forces under investigation. The recording system reacts to movement in a definite direction (usually in the head to foot direction) of the centre of gravity of that part of the body—in our case the thorax—which is on the recording table of the system. This is determined by the circumstance that in our arrangement of the gauges the recording system is sensitive to a change of moment of a vertical force, or to the vertical component of all the forces acting on it.

The oscillographic recording is carried out at the present time by means of an apparatus produced to our design by the Experimental Workshop of the Academy

of Medical Sciences of U.S.S.R. The apparatus includes two amplifying channels intended for the investigation of mechanical processes with the aid of the strain gauges of ohmic resistance, and three amplifying channels for the recording of the electrocardiogram. One of these electrocardiographic amplifying channels is adapted for the recording of the phonocardiogram. With the aid of one of the amplifying channels for the registration of the mechanical processes we investigate the thoracic dynamocardiogram with the vibrations under investigation directed along the long axis of the body. The other amplifying channel for investigation of mechanical processes is used for the recording of the changes of pressure in the cavities of the heart and large vessels of the sphygmogram of the carotid and other arteries, of the phlebogram, of the oesophagocardiogram, the ballistocardiogram, or the dynamocardiogram, but not in the longitudinal but in the transverse direction, or not from the thorax but from other parts of the body.

In normal quiet breathing the apparatus records not only the heart contractions but also the respiratory movements. If the subject holds his breath, or the breathing effect is suppressed electrically, only the heart's activity is recorded. The curves so obtained—dynamocardiograms—are quite complicated, and our first task has been to interpret them.

The dynamocardiograms of healthy subjects are characterized by a fairly high constancy of form of curve and of duration of its individual components, or of their time intervals. A typical normal dynamocardiogram is shown in Fig. 2.

In dynamocardiograms from healthy subjects, particularly of those of youthful age, and engaged in physical work or sport, there are often seen curves differing somewhat by the presence of accessory small peaks.

We interpreted (or, in other words, analysed physiologically) the dynamocardiogram by making a comparison between it and the simultaneously recorded electrocardiogram, the carotid sphygmogram, the pressure curves of the heart cavities, and

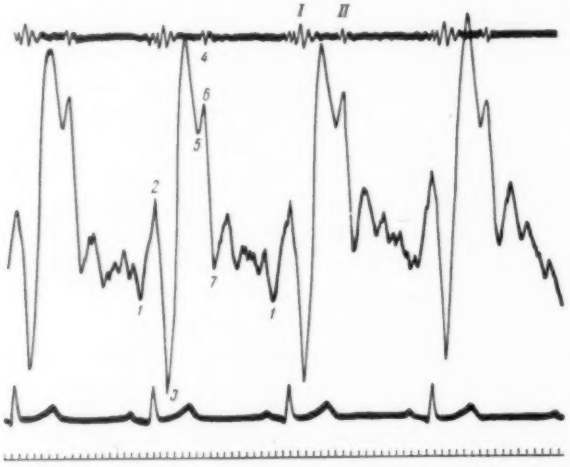

Fig. 2. Longitudinal thoracic dynamocardiogram of a healthy subject recorded simultaneously with the electrocardiogram and phonocardiogram. Time signal 0·05 sec.

of the pulmonary artery (by catheterization in man), together with the phonocardiogram and the phlebogram.

Without going here into a detailed physiological analysis of the dynamocardiogram (this we have done in a special article [2]) we will point out that this curve (Fig. 3) reflects all the phases of the cardiac cycle;

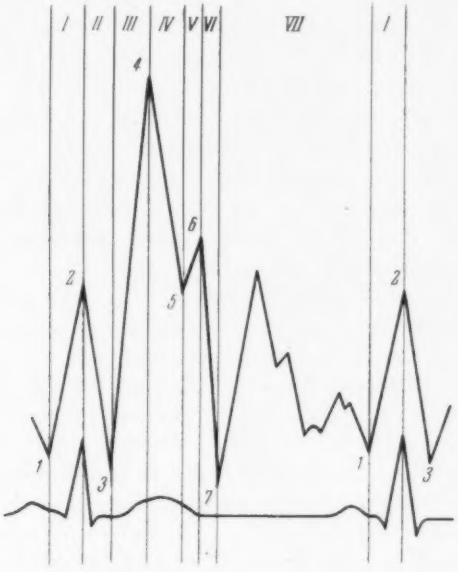

FIG. 3. Diagram of the dynamocardiogram. The spikes on the curve are indicated by Arabic numerals; the intervals by Roman numerals. Interval I systole of atria; II phase of isometric excitation of ventricles; III phase of sudden ejection of blood from ventricles; IV phase of slow ejection of blood from ventricles; V protodiastolic period; VI phase of isometric relaxation of ventricles; VII phase of filling of ventricles.

auricular systole; isometric pressure phase in the ventricles, the phases of rapid and slow ejection of their contained blood, and the phase of their filling. The moments of opening and closing of the valves are also reflected in the dynamocardiogram. Besides this, the accessory spikes on the curves, sometimes seen in the normal, and as a rule in the cardio-vascular disease, make it possible to determine a lack of synchromy in the work of the right and left ventricles. This we were able to confirm by other methods of investigation—X-ray cinematography, sphygmography of the carotid artery, recording of pressure variations in right ventricle and pulmonary artery.

In completeness of information on the phases of the cardiac cycle our method has an essential advantage over all other methods of investigation, as they give only partial data.

In this connection it should be mentioned that the dynamocardiograph is quite distinct from the ballistocardiographic technique, not only with regard to the physiological significance of the individual spikes of the curve it records, but also in regard to the nature of the physical processes reflected in the curve, and finally in the results

of its clinical application. Ordinary ballistocardiography records the displacement or speed or acceleration of the body along its long axis produced by the reactive "recoil" resulting from the ejection of the blood into the main vessels, and its movement in the vascular system.

Simple experiments showed that displacement of the whole body and the phenomena depending on horizontally directed forces (in the reclining subject) are not recorded on the dynamocardiogram. We recorded simultaneously on the same band of photographic paper two dynamocardiograms: the usual one, which we call the thoracic, and the abdominal or femoral. For this purpose we mounted two identically orientated receivers on the subject's couch. The first was situated, as usual, beneath the thoracic cavity, the second beneath the abdominal cavity or even beneath the thigh and the upper third of the shin. If the curves recorded by the receivers depended on a displacement of the body caused by forces in the horizontal direction all the peaks in both curves should be identical, and each should be deflected to one and the same side, in one and the same direction.

The experiments showed in actual fact that the thoracic and femoral records are different, and most important—that the main deflection of the beam of the oscillograph in the thoracic dynamocardiogram, corresponding to the phase of rapid ejection of the blood from heart to aorta and pulmonary artery, is directed upwards, while in the abdominal or femoral dynamocardiogram the main deflection is directed downwards (Fig. 4). This means that the main deflection of the beam is in the same direction as the blood stream; in the thorax in the direction of ejection of the blood from the ventricles, i.e. in the head direction, and in the abdominal cavity or the thigh in the direction of the blood stream in the abdominal aorta and in the femoral artery, i.e. towards the feet. Not only the main deflection, but all the remaining peaks of the dynamocardiogram reflected in the abdominal and femoral curves are oppositely directed to the peaks of the thoracic dynamocardiogram.

This convincingly proves that our technique and the ballistocardiogram record different mechanical processes connected with heart activity. This is indeed also shown by the difference of the configuration of the curves obtained by the two methods.

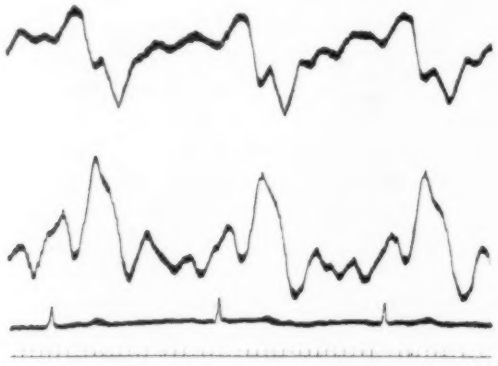

FIG. 4. Simultaneous records of thoracic (upper) and abdominal (lower) dynamocardiogram.

The origins of the physical processes causing the oscillations recorded by the dynamocardiogram have been considered in a special paper [3]. Without therefore going into this question we will content ourselves with the observation that the dynamocardiogram records displacements of the partial centre of gravity of the body, which depend primarily on the displacements of masses in the body, i.e. on the displacements of the mass of the heart and of the blood, and partly on the heart and pulse beats. That our method can record displacements of masses in the body is shown by a record of the transfer of water along the oesophagus. If the subject, lying on the recording apparatus, swallows some water, its movement along the oesophagus is clearly reflected in the record (Fig. 5).

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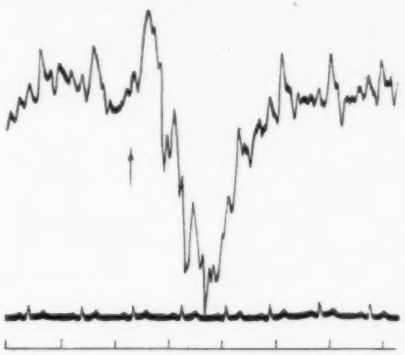

FIG. 5. Curve recorded during swallowing. Time signal 1 sec.

We have carried out a detailed analysis of the time relationships of the cardiac cycle in healthy subjects and in various types of cardiac disease, and on the basis of the investigation of 100 normals have calculated the average duration of each of the seven phases of the cycle (see table). The duration of each phase depends in greater or lesser degree on the rhythm of the heart beat. An even closer dependence on the rhythm is shown by the period of filling of the ventricles: this for a rapid cardiac

AVERAGE DURATION OF THE PHASES OF THE CARDIAC CYCLE
(Based on investigation of the dynamocardiograms of 100 healthy subjects)

Phases of the cardiac cycle (intervals on the dynamocardiogram)	Limits of variation at cardiac rhythms from 45-120 beats/min sec	Average values (sec)
Ventricular tension (interval II)	0·05–0·10	0·074 ± 0·001
Rapid ejection of blood from ventricles (interval III)	0·07–0·15	0·112 ± 0·002
Slow ejection of blood from ventricles (interval IV)	—	0·116 ± 0·002
Ventricular systole (intervals II–IV)	0·22–0·37	0·301 ± 0·003
Protodiastolic period (interval V)	0·03–0·045	0·032 ± 0·001
Isometric relaxation (interval VI)	0·07–0·11	0·093 ± 0·001
Filling of ventricles (interval VII)	0·11–0·80	0·376 ± 0·015
Systole of atria (interval I)	0·05–0·11	0·078 ± 0·001
Total cardiac cycle (intervals I–VII)	0·5–1·32	0·894 ± 0·016

rhythm (100/min) averages 0·11 sec and for a slow rhythm (45–50/min) 0·80 sec. The duration of the systolic phase is considerably less affected by the rhythm. Thus the duration of the isometric phase of the ventricles falls within the limits 0·05–0·10 sec, and the phase of rapid ejection of blood between 0·07 and 0·15 sec. The most constant time intervals are those of the protodiastolic period, amounting at various rhythms to 0·03–0·04 sec, and that of isometric relaxation, 0·07–0·11 sec.

Analysis of the numerical data led V. L. Karpman to a series of quantitative laws governing the time relationships within the cardiac cycle. Thus the dynamocardiographic data showed that there is a definite relationship between the total period of ejection of blood from the ventricles and the total period of their systole. This relation is termed the endosystolic index; it does not depend on the rhythm of the heart beat, and has a constant value in healthy subjects of 75 per cent, i.e. the ejection of blood occupies 75 per cent of the whole ventricular systole. The value of the systolic index is diminished in some pathological conditions of the heart, for example in cardiosclerosis and severe forms of mitral stenosis. On the other hand in athletes in training, the endosystolic index can rise to 80 per cent or even to higher values after physical effort.

On the basis of our numerical data we analysed the temporal relationships of the electrical and mechanical phenomena of cardiac activity, i.e. of the electrocardiogram to the dynamocardiogram. Apart from its purely theoretical interest, the investigation of these relationships has also a certain amount of practical importance in the clinic, inasmuch as in an incipient pathological condition of the heart there is a prolongation of the time interval between the electrical and mechanical manifestations of the heart beat. Calculation from simultaneous records of electrocardiogram and dynamocardiogram in 30 healthy subjects showed that the interval from the beginning of the ventricular complex of the electrocardiogram, i.e. from wave Q to the beginning of the phase of isometric tension, i.e. to peak 2 of the dynamocardiogram (Fig. 3), amounts to 0·02–0·07 sec with an average of 0·047 sec. It is worth while to recall the complete agreement of the mean interval between the electrical and mechanical manifestations of the heart's action as found by us and by a group of Italian workers [4]. In their investigations, published in 1954, they applied to man a technique of recording blood pressure in the ventricles analogous in principle to that used in Chauveau and Marey's experiment on the horse in 1861. In this method there was introduced into the cavity of the heart a sound terminating in a rubber balloon, the changes of pressure in which were recorded manometrically. A computation of the interval between the apex of spike R and the initial phase of the ventricular tension showed the average length of this interval was 0·013 sec with variations from 0–0·04 sec. The termination of the electrical systole, i.e. the termination of wave T of the electrocardiogram, coincides with the first period of diastole—the protosystolic period, which is represented on the dynamocardiogram by the interval between points 5 and 6 (Fig. 3). The lapse of time from point 5, when the ejection phase of the ventricles ends, i.e. when mechanical systole ends, to the end of the T-wave, i.e. to the end of electric systole, amounts to 0–0·06 sec, with a mean of 0·017 sec. Thus electric systole—the $QRST$ complex of the electrocardiogram—begins earlier and ends later, i.e. lasts longer than the mechanical systole. It follows from this that the duration of the $QRST$ complex does not give an accurate measure of the length of ventricular contraction.

The duration of the electrical systole of the ventricles, or period occupied by the *QRST* complex, bears a definite relation to the duration of the cardiac cycle, that is, to the *R-R* interval. A large number of different empirical formulas have been put forward to express this relation, the most widely accepted of these formulas consisting of the equation of the straight line $S = mC+n$, or of that of the parabola $S = K\sqrt{C}$. In these formulas S is the duration of the electric systole of the ventricles, C the duration of the heart cycle; m, n, and k are coefficients whose values differ considerably according to the results of various authors. For the parabolic equation the suggested values of K vary from 0·37 to 0·40.

Since the electric systole is somewhat more prolonged than the mechanical the formulas calculated for the duration of the former are not applicable for the expression of the latter. Further, two formulas proposed for the empirical calculation of the duration of the mechanical systole at different rhythms of heart beat are incorrect. The first of these is that proposed by Lombard and Cope in 1919 [5] and has the form:

$$S = \frac{60}{K\sqrt{R}}$$

where S is the duration of mechanical systole; R is the cardiac rhythm and K a constant equal, for the reclining human subject, to 25. This formula was worked out on the basis of data obtained by recording the carotid pulse: it is inexact because the sphyglogram of the carotid artery does not reflect the phase of isometric tension and the protodiastolic period, and, consequently, does not permit of a more or less accurate determination of the duration of the mechanical systole of the ventricles. The second formula was suggested in 1954 by de Simone, Cucei and Maselis [6], and has the form:

$$S = 0{\cdot}24T\sqrt{C}.$$

It is calculated from the results of the ballistocardiograph and makes it possible apparently to calculate the duration of the ejection phase but not of the whole of the systole.

On the basis of the computation of the dynamocardiograms of 100 subjects V. L. Karpman, together with ourselves, came to the conclusion that the relationship between the duration of mechanical systole and that of the whole cardiac cycle could be best expressed by the formulas:

$$S = 0{\cdot}183C+0{\cdot}142 \text{ and } S = 0{\cdot}324\sqrt{C}.$$

Calculations of the duration of mechanical systole in the human heart by these formulas give a good agreement with direct dynamocardiographic records. The divergence between calculated and directly observed values does not exceed 0·005 sec, and so falls within the limits of experimental error (Fig. 6).

From the formulas relating the length of electrical and mechanical systole to the duration of the cardiac cycle, it is not difficult to determine the time relation between the mechanical and electrical manifestations of cardiac activity [2]. This relation, which we propose to call the mechano-electrical coefficient of the heart (K m.e.), is in fact equal to

$$K \text{ m.e.} = \frac{S \text{ mech.}}{S \text{ electr.}} = \frac{K \text{ mech.}\sqrt{C}}{K \text{ electr.}\sqrt{C}} = \frac{K \text{ mech.}}{K \text{ electr.}}$$

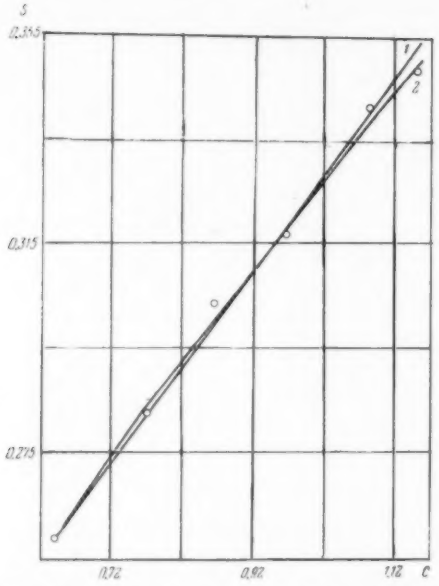

Fig. 6. Graph relating time of contraction of the ventricles to duration of cardiac cycle. Straight line 1 represents the formula $S = 0{\cdot}183C + 0{\cdot}142$. Curve 2 represents the formula $S = 0{\cdot}324\sqrt{C}$. The points are average figures of direct determinations with the dynamocardiograph.

The value of this coefficient amounted in our results to 0·868. This means that the duration of the mechanical systole, if expressed as a percentage, comprises about 87 per cent of the duration of the electrical systole.

Investigating further the relation between the manifestations of cardiac activity here under study, we found an expression for the difference between the durations of electrical and mechanical systole of the ventricles in terms of the duration of the whole cardiac cycle. It is:

$$S \text{ electr.} - S \text{ mech.} = 0{\cdot}05\sqrt{C}.$$

A special part of our work consisted in the vector analysis of the dynamocardiogram. By placing on the elastic elements of the recording arrangement a double number of gauges with ohmic resistance, and joining the second set into a special bridge circuit, it is possible to investigate the mechanical processes associated with cardiac activity and manifested by a displacement of the partial centre of gravity of the body, not only in a longitudinal but also in the transverse direction. Recording simultaneously both these processes on the cathode-ray tube, which constitutes an automatic integrating system, it is possible to observe the vector of displacement of the centre of gravity of the thorax or abdominal cavity as determined by the direction of contraction of the heart and the flow of blood in the vessels. This was achieved by Akulinichev and ourselves [7] in the following way: we led the signals from the dynamographic gauges from the long axis of the body to the vertical deflecting system and those from the transverse axis of the recording system to the horizontally deflecting system. The oscillations from longitudinal and transverse axes of the body

respectively could thus be separately recorded. Simultaneous recording from both pairs of deflecting plates gives a deflection of the beam determined by the magnitude and direction of the vector. As the result of the forces acting changes continuously throughout the whole cardiac cycle, the electron beam traces on the screen of the oscillograph a complex curve—the vector dynamocardiogram. As follows from what has already been said, this curve is the projection on to a plane of the resultant sum of the mechanical processes bound up with cardiac activity and the movement of blood in the vessels of the part of the body investigated. In order to analyse this curve in each experiment we recorded the dynamocardiogram in each direction separately and then the stationary vectorogram and its unfolding along a horizontal axis (Fig. 7). The analytical reconstruction of the vector from the simultaneous record

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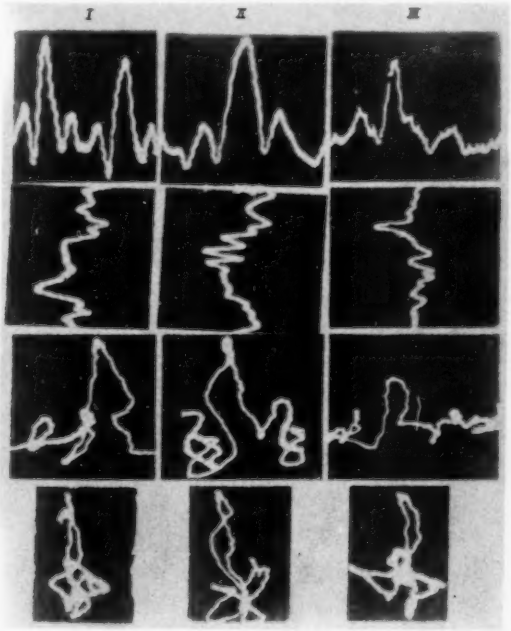

FIG. 7. Dynamocardiograms of one cardiac cycle in each of three healthy subjects (I, II, III) in the prone position. Upper row: dynamocardiograms corresponding to the long axis of the body. Second row: dynamocardiograms corresponding to transverse axis of the body. Third row: vector dynamocardiograms unfolded in the horizontal direction. Lowest row: vectorograms without artificial unfolding.

of the longitudinal and transverse dynamocardiograms made by the two bands of one and the same oscillograph made it possible to approach the interpretation of the vectorogram.

With the recording apparatus below the thorax of the prone subject we found that the vectorogram had three loops: (1) an initial loop corresponding to auricular systole and the tension phase of the ventricles, (2) a loop of maximum amplitude corresponding to the period of ejection of blood from the ventricles, and (3) a final

loop corresponding in time to the period of relaxation and filling of the ventricles. The initial loop is at the base of the vectorogram and possesses a complicated configuration. The loop of largest amplitude is directed upwards, and at its apex it has a small flourish. The final loop is directed towards the left half of the thorax. We suggest that the rising loop is caused mainly by the rejection of blood into the aorta.

By recording vectorograms with the subject lying not only on the back, but also on the right or left side, it was possible to carry out not only plane, but also three-dimensional vector analysis of the displacements of the partial centre of gravity of the body caused by heart activity.

If the vectorograms are taken with the recording apparatus beneath the subject's abdomen a considerable simplification of their form is discovered; the horizontal loop is almost completely absent from the thoracic dynamocardiogram depending on the activity of the heart, and only the vertical component in the direction of the blood flow in the abdominal aorta appears (Fig. 8).

Fig. 8. I: Vector dynamocardiogram recorded with the subject lying on the left side; II: on the right side; III: on the back.

We have so far made little use of vector analysis in the clinic, but the isolated observations that we present show that under pathological conditions when there is a severe interference with the normal control of the blood stream as, for example, in Fallot's tetrad, the vectorogram shows very great changes. As an example, we show the vectorogram of a patient in whom a patent Eustachian valve was diagnosed and a valvular construction of the pulmonary artery and a right-sided aorta (Fig. 9).

In contrast with the vector dynamocardiogram, which has been but little studied clinically, the ordinary dynamocardiogram has been widely used for clinical investigations for diagnostic purposes, for the appraisal of the effectiveness of therapeutic measures, and for the analysis of certain problems in clinical pathology. It is satisfying to be able to say that the investigation of the mechanical manifestations of cardiac activity by our technique has now completely justified itself [8–12].

As we cannot deal with this question in detail we will consider only a few results

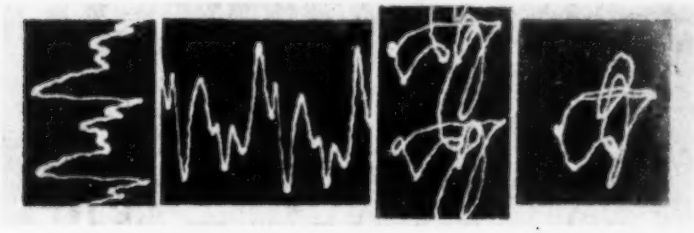

FIG. 9. Dynamocardiograms of the cardiac cycle in Fallot's tetrad, with right-handed aorta. Symbols as in Fig. 7.

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obtained in the surgical clinic directed by A. N. Bakulev on patients suffering from mitral stenosis. Of this pathological condition there are found to be three types, distinguished by their dynamocardiographic records. These we denote as the first, the intermediate, and the second types of characteristic change in the recorded curve in mitral stenosis (Figs. 10–12). The main difference between the first, intermediate, and second types of dynamocardiogram in mitral stenosis consists in the difference of amplitude relationship of the curve in intervals II and III, i.e. in the period of isometric tension and sudden ejection of blood from the ventricles. Analysis of the results in comparison with the clinical findings led us to the conclusion that the nature of the change in the dynamocardiogram depends on the degree of derangement of the blood circulation in the pulmonary circuit (V. L. Karpman).

The dynamocardiograms of patients with mitral stenosis are distinguished by a number of characteristic peculiarities. One of these is the great prolongation of the phase of isometric tension (reaching in this disease to 0·15 sec) and the disruption of

FIG. 10. Longitudinal thoracic dynamocardiogram in a case of mitral stenosis. First type of changed curve. Simultaneous records of electrocardiogram in leads I and III and phonocardiogram. Time signal 0·05 sec.

Fig. 11. Longitudinal thoracic dynamocardiogram of a case of mitral stenosis. Intermediate type of changed curve.

the curve in this phase, i.e. the appearance of a secondary peak on the dynamocardiogram during this period. Simultaneous recording of the dynamocardiogram and the sphygmogram of the carotid artery showed that the anocratic rise of the pulse wave begins at the moment of the appearance of the extra wave on the dynamocardiogram during isotonic tension. On recording the blood pressure in the right ventricle and the pulmonary artery by introducing into them a catheter carrying an electromanometer it appeared that the ejection of blood from the right ventricle goes on until the sharp fall of the curve, which we usually regard in the normal as the end of the phase of tension in the ventricles. On the basis of these findings we can state that in mitral stenosis there is a disturbance of the synchronism of the left and right ventricles, which

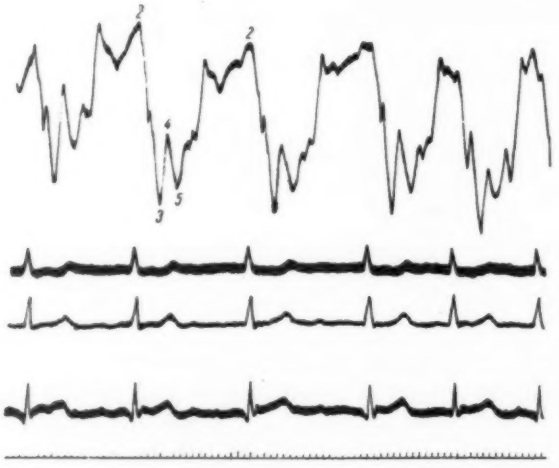

Fig. 12. Longitudinal thoracic dynamocardiogram of a case of mitral stenosis—second type of changed curve.

is discovered by the technique of dynamocardiography. The tension phase of the left ventricle has its normal duration, but that of the right ventricle is considerably prolonged. The reason for the asyncrony of the heart beat lies in the smaller power of the right ventricle to work against a considerably higher pressure than normal in the pulmonary vessels by a supernormal resistance in the lesser circulation as a result of stasis; the result is a prolongation of the tension phase of the right ventricle.

In connection with what has been discussed we will remark that we noticed asynchrony between the right and left ventricles also in other pathological states. It is typical that the delay was observed predominantly in the right ventricle with a relatively normal duration of the phases of the heart cycle in the left ventricle. As a result, the intrasystolic index in the left ventricle is more stable than that in the right.

To express the disturbance of synchrony of contraction of right and left ventricles it seems to us appropriate to use the term "asynchronia", which, as denoting a symptom of definite pathological phenomena, has the same justification as the term "arhythmia".

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The above findings have not only a theoretical interest but also a direct practical significance, inasmuch as dynamocardiography permits a refinement of the diagnosis of mitral stenosis. A great importance attaches to the determination of the types of disturbance of the dynamocardiogram in this disease. Comparison of the types of change of our curve with the results of mitral commissurotomy carried out in more than a hundred cases showed that the post-operative mortality is different in patients with the various types of changed dynamocardiogram. Thus, with the first type of change the mortality after operation was 4 per cent; in the intermediate type the figure grows to 24 per cent, and in the second type it reaches 40 per cent. This makes clear the importance of dynamographic pre-operative investigation of these patients.

Our technique gives, in addition, quite definite results for the appraisal of the effectiveness of the operative interference and of other therapeutic measures, in particular of pharmacotherapy.

The material we have presented in this article convinces us that the investigation of the mechanical manifestations of cardiac activity and the elaboration of methods for their investigation have no small importance for the physiological analysis of cardiac activity and disease.

Translated by T. PARSONS

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INVESTIGATION OF VARIOUS STAGES IN THE CONTRACTION OF CROSS-STRIATED MUSCLES BY ELECTRON MICROSCOPY

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(*Received* 1 *August*, 1956)

THE changes in the striated structure of muscles on contraction, and hence the role of this structure in the contraction process, have for a long time been of great interest to research workers of various special interests. Contradictory results were obtained in the older work on this question, according as to whether fixed or living material was observed.

The classical investigations on fixed preparations showed [1, 2] that the ratio between the isotropic and anisotropic discs changed on contraction of the muscle in the direction of a relative increase of the anisotropic.

Contrary to this, Hürthle [3], who investigated the contraction of living muscle fibres of insects, using cine-photomicrography and subsequently projecting the photographs, found that the height of the anisotropic disc (A) was considerably reduced by contraction, while that of the isotropic ones (I) remained unchanged. He concluded that the active contractile element was the anisotropic part, the isotropic part being passive. The difference between the data earlier obtained with fixed material, and the data he had obtained, Hürthle explained as due to artefacts produced by fixing. This point of view is strengthened by the following observations: in fixed preparations even of resting muscle, the anisotropic disc was always larger than the isotropic in comparison with measurements on living muscle fibres.

Frank [4] attempted to eliminate the contradictions between the data for fixed and living material by stating that the concept of the resting condition was inapplicable to preparations fixed by the usual methods, since in this case there was always present some degree of contraction, due to the stimulating action of the fixative. The excitability could be reduced by slow cooling or by fatigue up to complete exhaustion, which appeared to make it possible to obtain fixed preparations with an I/A ratio corresponding to that observed *in vivo*. Thus it was shown that in fixed preparations some types of contracture and contraction caused in fatigued muscle by the fixative could be obtained without so considerable a relative increase in disc A with respect to disc I. It was concluded that the old classical data were not fixation artefacts in the true sense of the word, but reflected a definite functional state of the muscle, caused in particular by the action of the fixative.

This question was later rendered still more complicated by some further results, analogous to those of Hürthle, that were obtained on living muscle (Holz [5], Buchtal *et al.* [6]). Holz and Buchtal observed that on isotonic contraction the ratio of the isotropic to anisotropic discs increased, owing to the contraction of the anisotropic

disc. Buchtal and Lindhard [7] showed that, under isometric conditions, the height of the anisotropic disc was reduced almost to one-half, the isotropic discs increasing in length correspondingly.

Thus these studies left the morphological picture of contraction in just the same chaotic state: the roles of both the isotropic and anisotropic substances in the contraction process remained unclear.

Current investigations represent a new step forward in the study of the structural changes on contraction, being conducted at a higher stage of technical development in microscopy. Typical studies are those of A. F. Huxley and Niedergerke [8], and H. Huxley and Hanson [9], in which optical artefacts, due to the thickness of the specimen, were eliminated. These workers obtained similar results: the first paper dealt with living fibres from the frog, while in the second glycerol-extracted myofibrils of rabbit muscle, acted upon by ATP, were used.

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It was found that the width of the anisotropic disc did not alter up to a definite degree of contraction of the sarcomere (contraction not exceeding 30–35 per cent of the resting length). Meanwhile the size of the isotropic disc was also reduced, or in other words, the relative size of disc *A* as it were increased at the expense of disc *I*. Upon further contraction both the isotropic and anisotropic discs became smaller, as long as the contraction of the sarcomere was not such as to cause the reorientation in the transverse structure that is termed "inversion of the striation". The change in the relation of discs *A* and *I* noted above for isotonic contraction is, most of all, in accord with the older data obtained on fixed preparations [1, 2, 4].

Thus Hürthle, who was the first to put forward the view that the difference between the data from fixed and living specimens was due to fixation artefacts, himself, evidently, fell victim to optical artefacts when studying *in vivo*. This is also the case with the data of Buchtal *et al.* [6], as was pointed out in the papers indicated above.

In addition to the changes of discs *A* and *I* described above, which occur within physiological limits of contraction (not exceeding 30–35 per cent of the resting length), many workers have observed the phenomenon of "inversion of the striation" during strong isotonic contraction [10–14].

The morphological picture of this phenomenon is essentially the same, whether living or fixed material is studied. Inversion of the transverse striation shows itself as redistribution of the dense material of the anisotropic disc, and its concentration in the region of the membrane *Z*. Wide dense bands are thus formed, which stain deeply—the so-called contraction bands. As a result of their formation disc *A* loses its density, and the boundary between discs *A* and *I* vanishes.

There are in the literature many attempts to explain the nature and mechanism of formation of the contraction bands.

The hypothesis has been proposed by a number of workers that the contraction bands are formed by migration of a dense amorphous substance disposed within the anisotropic disc. This opinion was expressed by the earlier histologists, and has been confirmed by some recent investigations.

In 1933 Jordan [10] described the formation of the bands in detail. He considered that the anisotropic part of the sarcomere separates into two halves, each of which moves over to the line *Z*, so the contraction band is formed from two parts of anisotropic discs in adjacent sarcomeres.

Ashley [13], studying with the electron microscope dispersed muscle fibres that had been digested with trypsin, found that even when the isotropic disc and membrane were absent, on account of the digestion, the formation of somewhat similar contraction bands was not prevented. In that case the migrating substance accumulated at the ends of the anisotropic disc. According to Draper and Hodge [12], it is possible for material to migrate in the sarcomere: if we bear in mind that a muscular contraction lasts 0·002 sec, then the rate of migration must be equal to 0·2 mm/sec, which corresponds with rates of diffusion and electrokinetic movement.

The hypothesis that the contraction bands are formed by the migration of material is not accepted by all workers. Bennett and Porter [14], having compared sections of contracted and extended myofibrils in the electron microscope, and having performed simultaneous densimetric measurements, found an increased density of all the elements in the contracted myofibril, shown by an increase in the binding-power for osmic acid. But the increase in the densities of the membrane *Z* and the disc *I* is particularly large. The authors thus consider that the increase in the density of the isotropic disc, being much greater than for the anisotropic, reduces the contrast between *A* and *I*, thus producing the impression that the discs have disappeared. Rejecting the possibility that material may migrate within the sarcomere, they adopt the point of view taken by Meigs [15] that sarcoplasmic material may move into the myofibril. The translocation of the material may occur through *Z* or through *M*, it mostly accumulating around *Z*. The work of Edwards *et al.* [16] does not confirm Bennett and Porter's hypothesis.

Thus the question whether the contraction bands form by migration within the limits of the sarcomere, or in some other way, is as yet far from being answered.

The present work constitutes an attempt to study the dynamics of the changes in transverse structure during isotonic contraction by electron microscopy, giving special attention to the process of formation of the contraction bands form.

An electron microscope of high resolving power was used, as being a more accurate way of performing quantitative measurements on the alterations in the structure of the transverse striations. But the technique of the electron microscope involves fixing the specimen, and hence there are a number of difficulties common to all such investigations, particularly with such rapid processes as muscular contraction.

In attempting to study the processes of muscular contraction on electron-microscope specimens, we fixed the muscle fibres at different degrees of contraction, from which it was possible to establish the dynamics of contraction, by comparing a number of successive stages in the contraction of the muscle myofibrils.

METHODS

The solution of OsO_4 usually used to fix objects for the electron microscope penetrates relatively slowly into tissues and this restricts its application in the present work when fixation has to be carried out during active contraction—during electrical stimulation, for example. To achieve its object the fixative used must act rapidly, and must in addition preserve the structure well. A large number of the fixatives available do not satisfy these requirements. We used Carnoy's mixture, with an additional treatment with 1 per cent osmic acid, to give the objects high contrast.

As well as penetrating the organs, Carnoy's mixture preserves the structure well, particularly when thin muscle bundles are being fixed.

We used muscle bundles of diameter about 1·5 mm and length 15 mm, dissected from the sartorius muscle of the frog.

Muscle bundles at the resting length were separated from the whole sartorius muscle with a razor, and, without being cut off from the muscle, were attached to a thin glass or wooden rod. Only after they had been firmly attached were they cut off from the muscle. In this way bundles were obtained with the same lengths as *in situ*.

The structure of the contracted muscle was compared with that of the resting muscle. Since, as noted above, any fixative acts as a stimulus for muscular tissue, in order to obtain states approximating to the resting state, muscles that were only weakly excitable, or that had lost their excitability, were used. This was achieved by the strong fatigue caused by prolonged electrical stimulation, by the action of novocaine, or by extraction with 50 per cent glycerol at 0°C by Szent-Györgyi's method [17]. This extraction removes ATP, and the muscle can then no longer contract. Contraction can be restored by the addition of ATP in a salt medium of definite composition.

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The objects were prepared by the method described earlier [18].

Photographs were taken in a UEM-100 electron microscope at a magnification of 6200. The final magnifications were 18,600 and 12,400.

Sections were prepared on a microtome designed to cut ultra-thin sections (0·05–0·06 μ), and the embedding medium was dissolved away.

EXPERIMENTAL RESULTS

For fixing the muscle in a state of isotonic contraction we used stimuli that differed in the nature of their actions. Fixatives—Carnoy's mixture and osmic acid—were used as stimuli, as well as the electric current. They did not have identical actions on the muscle. Carnoy's mixture is a rapid-acting fixative, which causes a fairly sharp contraction of the muscle bundle at the instant of application. Osmic acid penetrates tissues more slowly, and has a gentler action on an excitable tissue, and in consequence it causes no initial sharp contraction. Apparently the result of its action is not so much a sharp phasic contraction, as a slow, gradual shortening of the muscle. This comparison on the one hand of fixative accompanied by electrical stimulation with fixative action alone enabled us to follow the transformation of the myofibril structure from the usual appearance of transverse striation at rest to that showing contraction bands, depending on the functional state of the muscle.

Fixation of the muscle bundle during electrical stimulation

The muscle bundle was fixed with Carnoy's fluid mixture during electrical stimulation at the moment of immersion into the fixative, at various degrees of contraction (from 1 to 40 per cent). The fact that the degrees of contraction of the muscle bundles were not the same made it possible to select sarcomeres with different degrees of contraction, and to follow in detail the changes in the anisotropic and isotropic discs during contraction.

Fig. 1 shows the transition from a myofibril in its resting state (our measurements show that the resting length of the sarcomere was 2·3–2·4 μ), through a number of

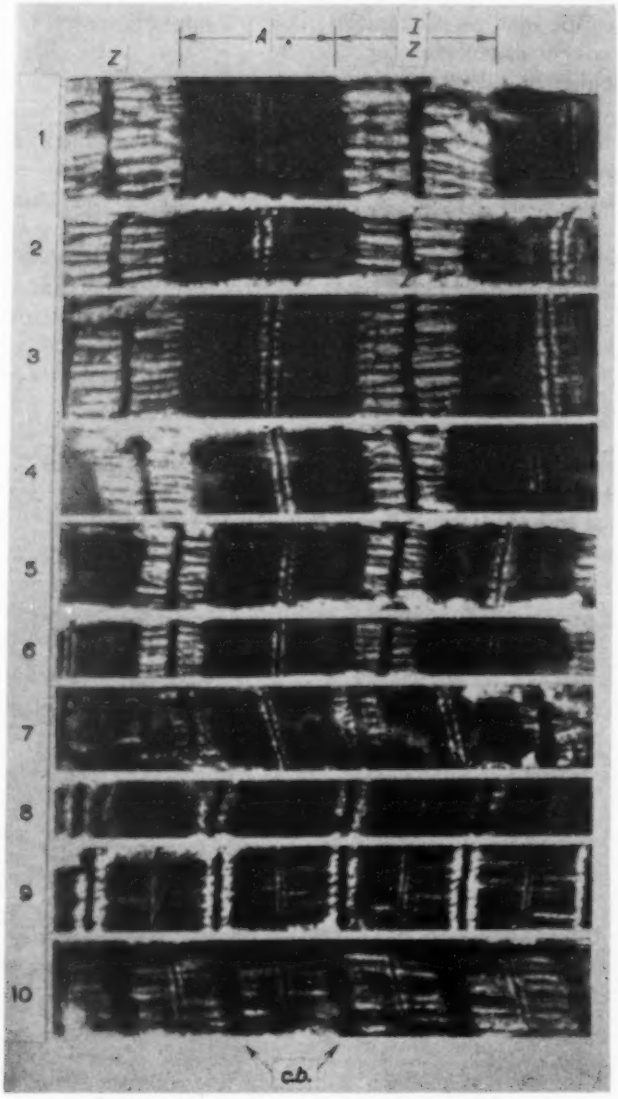

FIG. 1. Electron microphotograph showing a number of successive stages in the transition from myofibrils of resting length to contraction bands. The muscles were fixed with Carnoy's mixture while contracted under electrical stimulation. (1)—resting length; (2–9)—reduction in length of the sarcomere by 3, 10, 30, 35, 50, 55, and 58 per cent; (10)—reduction in sarcomere length reaches 60 per cent of the resting length; the contraction bands are seen (c.b.).

successive stages of sarcomere contraction, to the point where contraction bands appear. During this process the height of the anisotropic disc changes but little when the contraction of the sarcomere is small by comparison with its resting length. Only the isotropic disc is observed to contract. But starting at the point where the sarcomere has contracted by 20 per cent, the anisotropic disc also begins to contract. The contractions of the isotropic and anisotropic discs are compared in Fig. 2.

This photograph shows that the sarcomere has to contract by almost 60 per cent (i.e. more than to half length) before the contraction bands appear. A further contraction of the sarcomere after the contraction bands have formed was hardly to be observed.

We did not detect any stages intermediate between those where the discs were still plainly visible and those where the contraction bands were well developed. Apparently this transition to the final excessive contraction is extremely sharp when such an active contraction as that produced by the electric current is evoked.

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Contraction caused by osmic acid

Osmic acid acts slowly and gradually, and gives a picture of the contraction differing rather sharply from that given by electrical stimulation during fixation in Carnoy's mixture (Fig. 3). Here, as in Fig. 2, the transition from the resting length of the myofibril to the formation of contraction bands is gradual. The unusual action

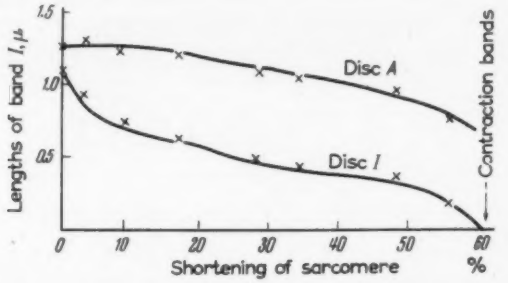

FIG. 2. Change of length of the *A* and *I* discs (ordinate) in relation to percentage contraction of sarcomere, from resting length (abscissa), on electrical stimulation (fixed with Carnoy's mixture).

of osmic acid apparently produces conditions such that the complex transformation of the myofibril does not occur so rapidly, and thanks to which a number of pictures of the transition from the stage with transverse striation to that with contraction bands could be obtained.

Figs. 3 and 4 show that during reduction of the total length of the sarcomere no contraction occurs in the anisotropic disc up to the point where it vanishes. Only the isotropic disc is seen to get smaller. It is interesting to observe that the formation of contraction bands here begins when the sarcomere has contracted only by 30 per cent of its resting length. The stages *D* and *E* shown in Fig. 3 are apparently transition stages on the way to the contraction bands. Here the disc *I* vanishes, and the anisotropic discs join up, but the contraction bands have not yet formed, and the dense substance is uniformly distributed throughout the whole sarcomere.

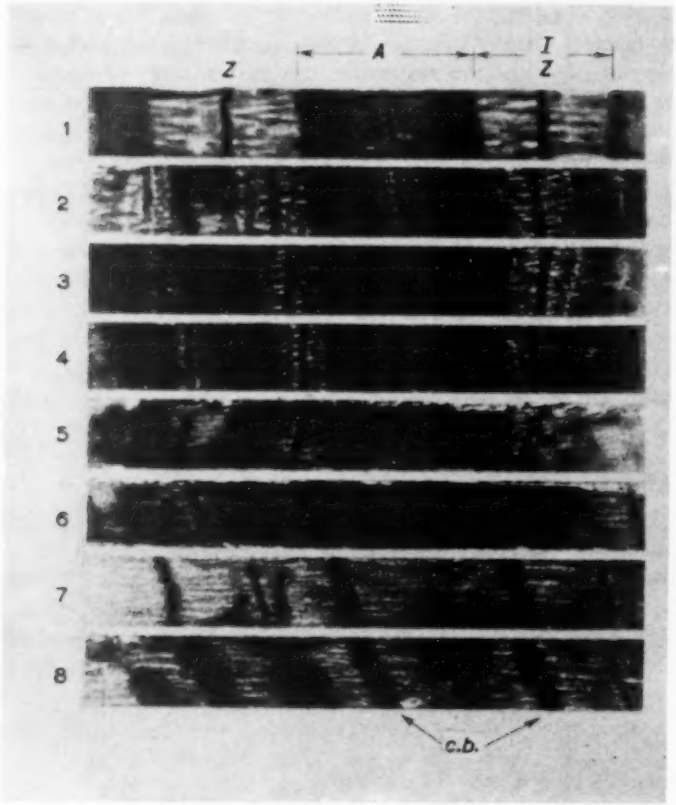

FIG. 3. Contraction of muscle fibres in osmic acid solution. Initial stages with formation of contraction bands. (1)—resting length; (2–4)—contraction of sarcomere from resting length by 10, 15, 20 per cent; (5)—contraction of sarcomere by 25 per cent, boundary between discs begins to be feebly transparent; (6)—contraction 30 per cent discs opaque, density uniform over whole sarcomere; (7)—contraction 40 per cent, contraction bands are still indistinct; (8)—contraction 44–45 per cent, contraction bands well marked.

After that, in the subsequent transitional stage *G* of Fig. 3, the density along the line of the membrane *Z* has increased considerably, and Fig. 3, *H* already shows well-developed contraction bands. It is also interesting to observe that the contraction of the sarcomere occurs in just the same way during the formation of the contraction bands as after they have appeared (their density then increasing).

Contraction in Carnoy's mixture

The changes in muscle bundles occurring on immersion in Carnoy's mixture without electrical stimulation occupy a position intermediate between those found for the action of osmic acid and of electrical stimulation. The length of the anisotropic disc in relation to the isotropic disc decreases inappreciably, and then mainly during those stages in the contraction which precede the formation of contraction bands.

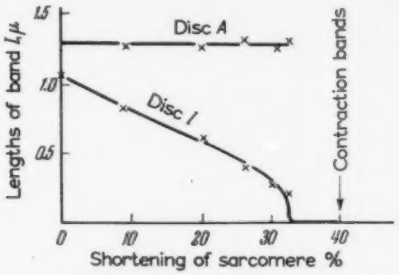

FIG. 4. Shortening of muscle fibres in a solution of osmic acid. The graph shows the relation between the lengths of the *A* and *I* discs (ordinate) as functions of the contraction of the sarcomere (abscissa), and also the point at which contraction bands are formed.

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Obviously, as a result of its stronger stimulating action on muscle, Carnoy's mixture causes a greater contraction of the sarcomere before the transversely striated structure is transformed, and the contraction bands appear. From Fig. 5, showing the relation between the degree of contraction in the sarcomere and the sizes of the anisotropic and isotropic discs, it is clear that the contraction bands appear when the sarcomere has contracted by 40 per cent of its resting length.

Like osmic acid, Carnoy's mixture causes a considerable contraction in the sarcomere even after the contraction bands have appeared.

Shortening of non-excitable muscles

We may suppose that the fixative has two actions which give rise to the observed effects not only of contraction of the sarcomere but also the concomitant transformation of the transverse striation—on the one hand, its action as a stimulus, and, on the other, its action as a denaturating and coagulating agent which may cause secondary changes in the structure under study.

In this connection it was possible to follow the reactions occurring in the transverse striations, when these same fixatives—stimuli—act on muscles whose excitability has been lost or very much reduced. At the same time, by rendering them non-excitable as regards the stimulating action of the fixative, it is possible to observe in a purer form the strict effect of the fixation. We produced a great weakening of the

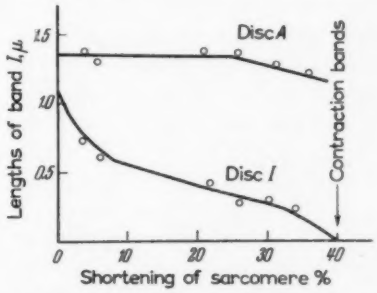

FIG. 5. Contraction of muscle fibres in Carnoy's mixture. Change in length of the discs *A* and *I* (ordinate) as functions of the percentage reduction in length of the sarcomere (abscissa).

excitability of the muscles by fatigue produced by prolonged electrical stimulation, and a complete loss of excitability by extraction of the muscles with 50 per cent glycerol in the cold, by Szent-Györgyi's method [17].

Muscles that do not respond to electrical stimulation should obviously not respond actively to the stimulating actions of Carnoy's mixture and osmic acid. Although the free muscle still contracts in the fixative, this contraction is of quite a different type, and should more accurately be termed contracture, and not a phasic contraction.

A completely different picture is in fact obtained with this passive contraction in the fixative of muscle whose excitability had been abolished or reduced.

In Fig. 6, (1) and (2) are compared; muscles that have been extracted with glycerol and fixed with osmic acid at their resting length ((1)*I*), and the same muscle contracted in osmic acid ((2)*II*). The difference as compared with living muscle is considerable, since the sarcomere here contracts as a whole by 40 per cent, while disc *A* contracts by 45 per cent, and *I* by 40 per cent. Not only does a complex structural reorganization not occur, accompanied by a redistribution of density within the sarcomere, but the relationship of the discs is as it were reversed: disc *A* contracts relatively more than disc *I*.

The picture obtained when Carnoy's mixture acts on fatigued muscle (Fig. 6, (2) and (3)), and on muscle that has been extracted with glycerol (Fig. 6, (5) and (6)), are also in essence analogous, but differ quantitatively in the values of I/A. A considerable contraction of the sarcomere (some 50 per cent) is not accompanied by a transformation of the transverse striation. All that can be seen is that the isotropic and anisotropic discs have independently become smaller, without an essential change of their relative heights.

DISCUSSION

We see from these data that fixation at the instant of electrical stimulation causes an unequal contraction of both discs, which is accompanied by the formation of contraction bands, only outside physiological limits. Formation of these bands alters the structure of the transverse myofibril striation, but does not lead to additional contraction in the sarcomere, since it has already contracted by 60 per cent before their formation.

In osmic acid the initial stages of contraction-band formation are seen when the sarcomere has contracted by only 30 per cent. At this degree of contraction, only the isotropic disc is seen to have shortened. The length of the anisotropic disc remains the same as in the resting state. But although the absolute length of disc *A* remains unchanged, its length relative to that of the isotropic disc increases as contraction proceeds. Approximately the same relation between the discs is seen on contraction in Carnoy's mixture.

However, Carnoy's mixture is clearly a stronger stimulant, since the contraction bands appear a somewhat greater degree of sarcomere contraction, i.e. 40 per cent. On the other hand, when electrical stimulation and fixative act together, the sarcomere contracts considerably even after the contraction bands have formed, with both osmic acid and Carnoy's mixture.

Hence any stimulus that causes contraction in living muscle also causes a change in the relation between the isotropic and anisotropic discs, which finishes by a

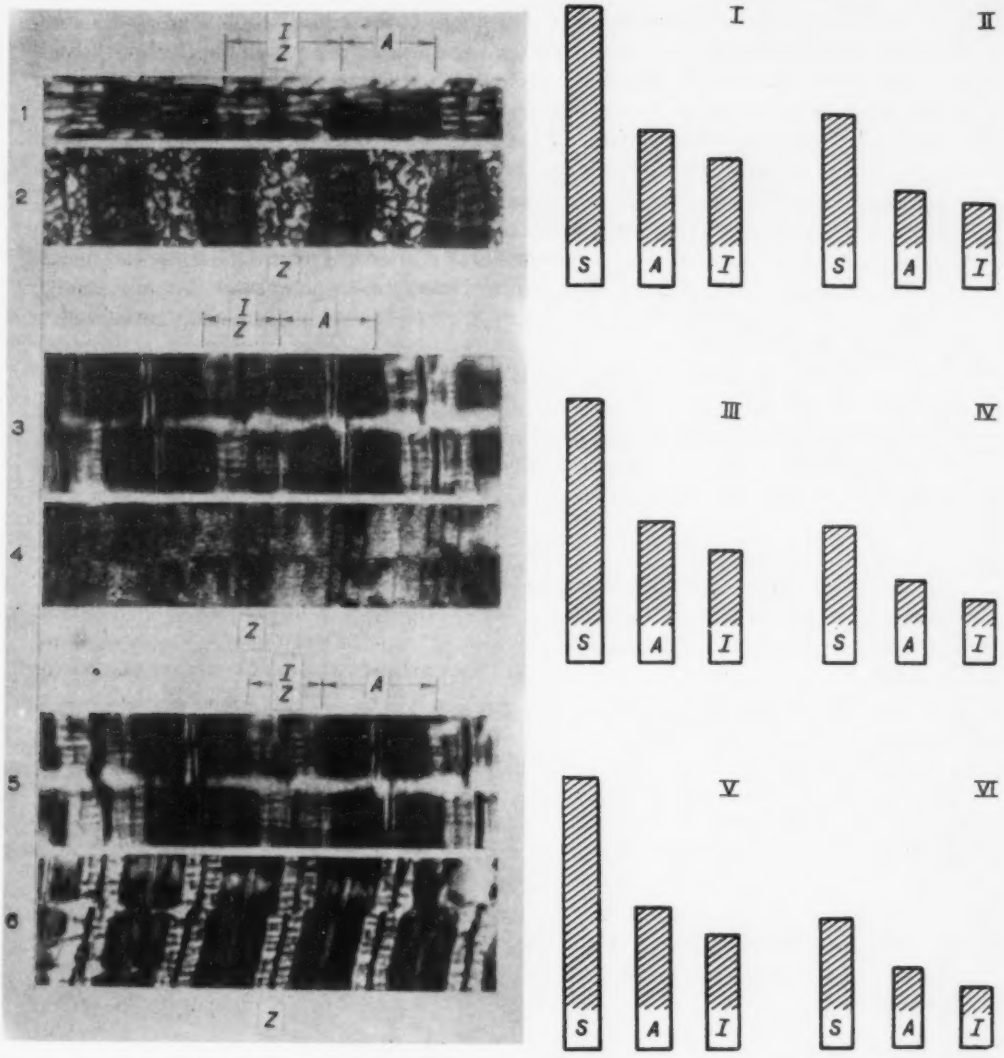

FIG. 6. Contraction of glycerol-extracted muscle in osmic acid: (1)—resting length; (2)—contraction of sarcomere reaches about 40 per cent, *A* being 45 per cent and *I* 40 per cent contracted. Contraction of glycerol-extracted muscles in Carnoy's mixture. (3)—resting length; (4)—whole sarcomere reduced in length by 50 per cent of the resting length (*A* by 40 per cent, and *I* by 60 per cent). Contraction of fatigued muscle in Carnoy's mixture. (5)—resting length; (6)—sarcomere contracted by 50 per cent of the resting length (*A* by 40 per cent and *I* by 60 per cent).

complex transformation of structure, i.e. by a redistribution of density in the sarcomere, and by the formation of contraction bands.

Essentially, however, the formation of contraction bands is not apparently rigidly connected with any definite degree of sarcomere contraction, since it occurs at different stages, depending on the nature of the action of the stimulus.

Further, when the muscle responds strongly to stimulation, as in electrical stimulation, a considerable degree of contraction—beyond the physiological limits—can occur without inversion of the cross striation. Conversely, when the fixative acts alone, and simultaneously stimulates and, obviously, poisons and denatures the muscle, the contraction bands form at low degrees of sarcomere contraction, and a greater degree of contraction is prevented by their formation.

The observed changes in the relations of the discs are apparently not artefacts, but are connected with a definite functional condition, which is also indicated by the experiments where the excitability is reduced or absent (glycerol extraction, fatigue).

In such muscles the characteristic features are either the absence of any change in the relation between the discs, or a change which is very slight as compared with the resting state.

No doubt in the latter case we are dealing with muscles which are capable of another form of contraction in other ways, in which the usual mechanism of redistribution of density in the sarcomere plays little part.

The author wishes to express her indebtedness to the team of the laboratory of electron microscopy in which the present investigation was carried out.

CONCLUSIONS

(1) The transformation of the striated structure (contraction bands) observed on isotonic contraction cannot, apparently, be correlated with any definite degree of sarcomere contraction. Depending on the nature of the stimulus, the contraction bands may be formed at 60 per cent contraction of the sarcomere (by fixation of the muscle at the instant of electrical stimulation), and at 30–40 per cent (by the stimulating action of fixatives alone).

(2) Depending on the nature of the stimulus, the contraction process itself may take place in different ways. While in the first case the sarcomere contraction mainly occurs before the formation of the contraction bands, in the latter case, on the other hand, it mainly occurs after the bands have formed.

(3) When the excitability is reduced or abolished, a considerable contraction of the sarcomere occurs without the formation of contraction bands, being brought about by independent contractions of the isotropic and anisotropic discs. This shows that the contraction bands form only when the normal functional state of the muscle is preserved.

Translated by J. E. S. BRADLEY

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THE DIFFRACTION PATTERN OF COLLAGEN

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(*Received* 30 *July* 1956)

VOL. 2 1957

THE study of fibrillar proteins of the collagen type is at the present time proceeding along two directions. The first is the determination from experimental data of a series of features of the configuration of the polypeptide chain; while the second involves the construction of models from theoretical considerations, with the addition of certain experimental data.

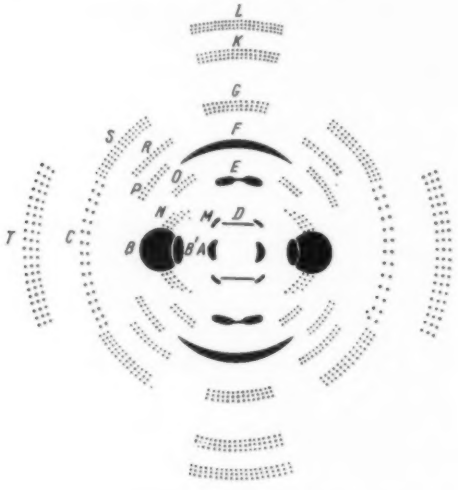
The determination of the features of the configuration of the polypeptide chain in proteins is, in the main, based on X-ray data. However, no complete X-ray photograph of collagen has so far been described in the literature, although the first were obtained as early as the 1920s.

No.	Reflection	*d* in Å	Intensity	Remarks
1	*A*	11 ± 2	Very strong	
2	*B′*	5·6 ± 0·1	Medium	
3	*B*	0·12	Medium	
4	*C*	2·1 ± 0·2	Weak	
5	*T*	1·6 ± 0·1	Very weak	
6	*D*	9·3 ± 1·1	Medium	Layer line
7	*E*	3·9 ± 0·2	Strong	At a certain angle between specimen and X-ray beam this splits up into two maxima falling on the main line
8	*F*	2·9 ± 0·1	Very strong	
9	*G*	2·0 ± 0·1	Weak	
10	*K*	1·65 ± 0·05	Weak	
11	*L*	1·42 ± 0·05	Weak	
12	*M*	7·6 ± 0·5	Medium	
13	*N*	4·9 ± 0·3	Weak	In this region of the photograph the layer lines are superimposed on a strong background
14	*O*	3·5 ± 0·2	Weak	
15	*P*	2·9 ± 0·9	Weak	
16	*R*	2·5 ± 0·8	Weak	
17	*S*	0·24	Weak	

Since that time a few studies giving additional data have been made. Thus, Randall reported the equatorial reflection with $d = 2{\cdot}15$Å [1]. Cohen and Bear [2] observed a number of meridional and near-meridional reflections, using Cu-radiation

and a cylindrical camera. Ramachandran [3] observed that the meridional interference with $d = 4$Å split up into two diagonal maxima when the specimen was inclined at a certain angle to the X-ray beam. Cohen *et al.* [4], using stretched collagen, obtained well-resolved photographs, and at the same time were able to show that the reflection with $d = 2{\cdot}9$Å increased to $d = 3{\cdot}1$Å.

But these isolated data do not give a complete picture of the collagen diffraction-pattern. In the present communication we have attempted to give a complete description of the diffraction-pattern for collagen from our own experimental data.* Well-orientated air-dried collagen (RTT, Rat-tail tendon) was used. The photograph was taken with monochromatic Cu-radiation, obtained by reflection from a crystal, using a flat and a cylindrical cassette and various angles of the specimen to the X-ray beam; and also with Mo-radiation at a specimen to plate distance of 70 mm, and again with the specimen inclined at various angles to the beam.



The observed reflections are summarized in the table, and in the schematic representation of the X-ray photograph (above).

It should be remarked that the reflections are not all identical in character. A diffuse maximum appears amongst them at

$$\frac{\sin\theta}{\lambda} \sim 0{\cdot}12.$$

In form it resembles the reflection corresponding to the packed chain of α-type proteins. The meridional reflection with $d = 9{\cdot}3$Å is a layer line: all other reflections may be ascribed to reflections from quasi-crystalline regions. The equatorial reflection with $d = 5{\cdot}6$Å may be considered as the second order of the reflection with $d = 11$Å. This latter reflection has a non-uniform intensity distribution along the equatorial line. The diagonal interferences with $d = 7{\cdot}6$Å, $d = 3{\cdot}5$Å, and $d = 2{\cdot}5$Å may be the first, second, and third order reflections for one and the same system of planes.

* The photograph was taken in the usual fashion, small-angle reflections being excluded.

The diagonal reflections *O*, *P*, *R*, and *S*, as judged by their diffuse texture, must be considered as composite.

Interest attaches to the comparison between the data so obtained, and those calculated from some models. Consideration of Ramachandran's Fourier transformation shows that there are a number of discrepancies between Ramachandran's model and the experimental data. Thus the X-ray photograph shows meridional bands *G* and *K*, which should not be meridional, according to the Fourier transformation. There is no maximum corresponding to the maximum *S* on the photograph in the Fourier transformation. The transformation does not explain the presence of the strong second-order reflection of the equatorial reflections with $d = 5{\cdot}6$Å.

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ON THE ROLE OF THE ACETYLCHOLINE-CHOLINESTERASE SYSTEM IN THE PHENOMENA OF GALVANOTAXIS AND SUMMATION OF STIMULI IN PARAMECIUM

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(*Received* 29 *November* 1956)

As early as the beginning of the present century the Moscow physiologist Statkevich [1], having investigated the phenomenon of galvanotaxis in protozoa, came to the conclusion that this peculiar form of reaction of protozoa to electrical stimulation is the result of a change of metabolism in the protoplasm, caused by the electric current, and not a physical phenomenon of cataphoresis as assumed by a number of other investigators [2]. This fruitful conclusion of Statkevich has received a number of experimental confirmations, mainly in investigations that have shown the dependence of the galvanotaxic reaction on various chemical actions in the protozoa.

In the work of Koshtoiants and co-workers [3] a whole series of facts was obtained that indicated the possibility of the action on the galvanotaxic reaction of a shifting of the course of metabolic processes, with a corresponding change of the excitability of the ciliate infusoria. Thus in the experiments of Popova [4] and Smirnova [5] it was shown that the sensitivity of *Paramecium caudatum* to the electric current falls on the binding of the active groups of its proteins and, in particular, of the sulphydryl groups. As blocking agents for sulphydryl groups, solutions of mercuric chloride,

cadmium chloride and S-S-glutathione were used. It was also made clear that on introducing into the culture substances containing free sulphydryl groups (cysteine, Bal) there occurs a restoration of the sensitivity of the Paramecia to the electric current temporarily lost by the blocking of the sulphydryl groups of the proteins. In addition it was shown in Popova's experiments that it was possible to restore the normal movements and galvanotaxis of Paramecia that had been disturbed by the action of potassium cyanide by the addition of succinic acid to the culture.

These results, which clearly indicate a dependence of the sensitivity of Paramecium to electrical stimulation on the state of metabolic processes, suggest a modern investigation of the presence and rôle in Paramecium of the various enzyme systems, among them acetylcholine-cholinesterase. The results of a whole series of investigations in comparative physiology indicate the functional significance of the acetylcholine-cholinesterase system in the production and conduction of excitation processes. In this connection a special interest attaches to presence and possible function of this system in nerveless organisms, and in particular in infusoria.

The question of the presence of the acetylcholine-cholinesterase system in the organism of infusoria, and in Paramecium in particular, was raised by Bayer and Wense [6] in their work of 1936. These authors came to the conclusion that the acetylcholine-cholinesterase system is present in Paramecium by testing the action of extracts of a culture of these organisms on eserinized dorsal muscle of the leech, and on the frog's heart. It should be mentioned that in our laboratory, Mitropolitanskaia [7] did not find acetylcholine in Paramecium or acetylcholine or cholinesterase in the parasitic infusoria of the goat's rumen. Bullock and Nachmansohn [8] similarly did not find the specific esterase in Paramecium.

Convincing evidence of the presence of cholinesterase activity of a homogenate of the infusorian *Tetrahymena geleii S.* was obtained in the work of Seaman and Houlihan [9] and Gerald and Seaman [10]. By fractionating a homogenate of this infusorian these authors succeeded in localizing the cholinesterase in the region of the pellicle at the base of the cilia, where there spreads a special fibrillar structure held by present-day cytologists to be concerned with the conduction of the excitation from one cilium to another. These authors also showed that anti-cholinesterase preparations depress the motility of Tetrahymena, from which it was concluded that the co-ordination of the movements of the cilia in these infusoria was dependent on the activity of cholinesterase.

We have set ourselves the problem of elucidating the physiological significance of the acetylcholine-cholinesterase system in the reaction of infusoria to electrical stimulation by investigations on galvanotaxis in Paramecium and the capability of summation of stimuli in this organism.

Statkevich first called attention to the ability of the protoplasm of Paramecia to summate actively sub-threshold electrical stimuli. He showed that, while single induced shocks did not affect the infusoria, a rapid succession of shocks directed them to the negative pole. Later the ability of infusorian protoplasm to summate electrical stimuli was investigated by the Japanese worker Kinoshita [11], who found that latent excitation rises rapidly at the beginning of a sub-threshold electrical stimulus, but more slowly towards the end of the stimulus.

In the analysis of our problem we considered that the action of anti-cholinesterase

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substances is not necessary confined to the specific action of cholinesterase, and in connection with this we investigated the action, not only of anti-cholinesterase substances, but also of acetylcholine, on the threshold for galvanotaxis and on the summation interval.

METHODS

Paramecium caudatum was cultured in an infusion of hay in Lozin-Lozinskii solution without peptone. The experiments were carried out by the technique earlier employed in our laboratory by Popova [4] and Smirnova [5]. The electric current was passed through a drop containing 20–30 individuals in the hollow of a microscope slide, by zinc-agar non-polarizable electrodes 6 mm apart. The animals were observed by means of a binocular magnifier ($17 \times 17{\cdot}5$).

Constant current of 10μA was taken from a battery of alkaline accumulators, the voltage across the electrodes being varied in the course of the experiments between 0·1 and 1 V. Interrupted current was obtained from a universal valve stimulator Gra-kh-I. The duration of each impulse was 5 /msec with an output voltage of 3 V. The frequency was 1 c/s and higher. In the control experiments the galvanotaxic reaction appeared usually at 10–11 c/s, which was taken as the norm.

In the experiments with constant current the threshold for the galvanotaxis was determined before and after the introduction of the substance into the drop containing the infusion. In experiments with interrupted current the frequency for galvanotaxis was determined in two parallel tests, the experimental with, and the control without, the substance under investigation. The thresholds taken were the minimal strength of the constant current, or the minimal frequency of the interrupted current, at which the animals began to swim towards the cathode. The duration of the action of the current on the infusoria was constant in each experiment (15–30 sec). The current was turned on every minute for equal periods of time.

The following substances were tried: proserin in concentrations of 3×10^{-4} to 3×10^{-15}; phosphochol in concentrations of 3×10^{-4} to 3×10^{-11}; acetylcholine in concentrations of 3×10^{-4} to 3×10^{-10}. These values refer to the final concentrations in the drop containing the infusion, and were made up in Lozin-Lozinskii medium without peptone. The same medium was used in the controls. In all solutions pH = 7·2–7·4 by the drop method with universal indicator and a series of buffer solutions.

RESULTS AND THEIR INTERPRETATION

Experiments on galvanotaxis showed that the anti-cholinesterase preparations phosphochol and proserin in small concentrations lower the threshold for the galvanotaxis. Phosphochol in the concentrations 3×10^{-11} to 3×10^{-8} lowers the galvanotaxic threshold from a norm of 10–15μA by 35–70 per cent with a later return to normal (Fig. 1, II). With higher concentrations of phosphochol the initial lowering of the threshold may be followed by a subsequent rise. With increase of concentration of the substance the rise of threshold becomes more marked, and may occur before the fall as well as after it.

In a concentration of 3×10^{-4} phosphochol produces only a rise of galvanotaxic threshold. The action of proserin is analogous to that of phosphochol. The difference consists in the sharper variations of threshold produced by proserin. In concentrations of 3×10^{-12} proserin lowers the galvanotaxic threshold. In all other concentrations

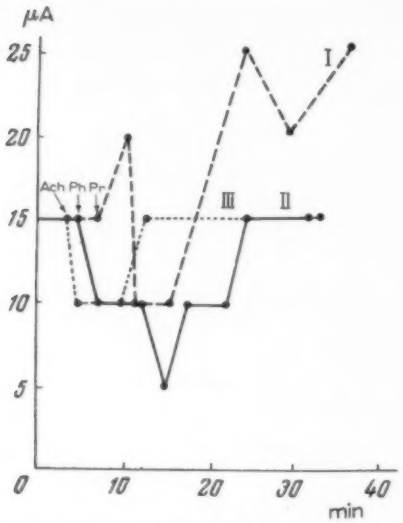

FIG. 1. Changes of galvanotaxic threshold of paramecia under the influence of proserin (3×10^{-9}), phosphochol (3×10^{-7}), and acetylcholine (3×10^{-7}). I—pr-proserin; II—ph-phosphochol; III—Ach-acetylcholine. Abscissa —time in minutes, ordinates—threshold in μA.

from 3×10^{-11} to 3×10^{-4} falls and rises of threshold may alternate with one another (Fig. 1, I), the falls of threshold being sometimes observed at those concentrations at which phosphochol usually produced only a rise. After a rise of threshold, a secondary lowering may be observed, but usually not reaching the normal.

The action of acetylcholine is similar to that of the anti-cholinesterase preparations (Fig. 1, III). In concentrations 3×10^{-7} to 3×10^{-8} it produced only a fall of galvanotaxic threshold with subsequent return to normal. In concentrations 3×10^{-6}, acetylcholine produced both a fall and a rise of galvanotaxic threshold. At concentrations 3×10^{-5} to 3×10^{-4} it produced only a rise of threshold. It should be remarked that in the control experiments the threshold was very stable and did not change during the course of the experiment (20–30 min).

Experiments on summation of stimuli

In the experiments with interrupted current the threshold frequency of the control was 10–11 c/s. Proserin in concentrations 3×10^{-8} to 3×10^{-9} caused a lasting fall of the threshold frequency to 2–3 c/s (Fig. 2). At higher concentrations of proserin the lowering of threshold frequency was less lasting and was replaced by a rise.

Acetylcholine in concentrations 3×10^{-10} to 3×10^{-9} also caused a lowering of the threshold to 2–3 c/s after which there usually followed a rise of threshold frequency. A small rise of threshold above the normal level may also be seen in experiments with proserin at concentration 3×10^{-5}. But this rise always occurred after a considerable lowering of the threshold (by 50–60 per cent).

From the data given it can be seen that the action of anti-cholinesterase preparations, and of acetylcholine, on Paramecia are similar to each other: in small concentrations (3×10^{-12} to 3×10^{-9}) they produce a lowering of the galvanotaxic threshold and a

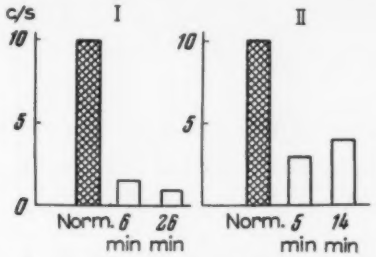

FIG. 2. Changes of summation interval in Paramecia under the influence of proserin. I—concentration of proserin 3×10^{-5}; II—concentration of proserin 3×10^{-4}. Abscissa—time of action of proserin, ordinates—threshold frequency cycles 1 sec.

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broadening of the summation interval; while in high concentrations (3×10^{-5} to 3×10^{-4}) they raise the galvanotaxic threshold and shorten the summation interval. In medium concentrations, with both anti-cholinesterase substances and acetylcholine, a lowering of the frequency and galvanotaxic thresholds may alternate with a rise.

CONCLUSIONS

The results obtained permit the conclusion that anti-cholinesterase preparations and acetylcholine in small concentrations raise the sensitivity of infusoria to the electric current. Increase of the concentration of these substances leads to the reverse reaction: the excitability is diminished and the sensitivity to the current falls. The parallelism between the actions of anti-cholinesterase preparations and acetylcholine itself supports the idea of an acetylcholine-cholinesterase system in Paramecium, and points to a physiological function of this enzymatic system in the irritability and sensitivity of protozoa. In general terms, it can be said that the enzyme system acetylcholine-cholinesterase, which plays an important part in various processes of nervous activity, and in particular in the phenomena of action potentials and electrical excitability in the nervous system, plays also a definite part at the pre-nervous level; and that the phenomena of galvanotaxis and summation of stimuli characteristic of Paramecia depend on its functional state.

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NATURE OF THE VARIATION OF THE BIOELECTRIC POTENTIALS IN THE REGENERATION PROCESS OF PLANTS

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(*Received* 10 *April* 1956)

THERE is an established opinion in biophysics that the bioelectric potentials of plant cells are simple derivatives of the metabolism, i.e. "waste products", not taking any active part in the processes of growth and development. However, it has been shown experimentally that the biopotentials reflect the level and intensity of metabolic, intra-cellular reactions [1–13], and are an inalienable sign of the phenomena of irritability [2, 5, 14–18]. The bioelectric potentials take an active part in the transport of growth-stimulating substances and water [5, 11, 19–24].

In theoretical investigations it has been assumed that an artificial application of an external e.m.f. to the tissue of plants does not produce any regular morpho-physiological variations [25]. On the other hand, practical experimental workers established that electricity has a stimulating effect on the growth and productivity of plants, and on the rate of maturing of the seeds. When an external potential is applied, the seeds of most species germinate more quickly than under the usual conditions. Electrization of the soil increases considerably the size of the roots [26–34]. External application of electricity has an effect on the differentiation of the cells, on the intensity of mineral nutrition, photosynthesis and respiration [3, 33–36].

Artificial application of an external e.m.f. on plant organisms is successfully used in selection work. Electrization of the soil in which the seedlings of experimental plants were raised, yielded in many cases markedly positive results in breeding new sorts of fruit trees with increased yield, high quality of the fruit, resistance to diseases and to low temperatures [30].

The literature also contains data on unfavourable effects of external application of electricity which led to the conclusion that electricity has a harmful effect on plants [29, 37]. The different character of the response of plants to the action of the same "dose" of an external e.m.f. is explained by the existence of the internal e.m.f.s of the plant cells, characterized by capacitance, potential, and polarity. The main difficulty in selecting the optimum doses of e.m.f. for achieving an intended effect seems to us to consist in the necessity of considering not only the magnitude and duration of the application of the external potential, but also the polarity of the e.m.f. of the plant cells. It has been found that stimulation of photosynthesis and accelerated growth of the tissues could be observed only in cases in which the internal electrical polarity of the organs of the plant was considered [5, 10, 20, 30, 38–42].

It is still impossible to draw final conclusions from the available data on the electrization of plants, since there is as yet no theory of the bioelectric potentials of plant

cells. On this question biophysics is only in the first stage of its investigations, viz. the qualitative generalization of practical and experimental results.

Since the facts, apart from their contradictory character, at least put the active influence of the potentials on the vital activity of the cells beyond any doubt, we decided to measure the bioelectric potentials in the initial stages of the regeneration of plants, i.e. during the process of deposition and development of a callus and also of a secondary meristem giving rise to additional growing apices. The question that interested us was whether there is a well-defined variation of the bioelectric potentials associated with the regeneration processes. If such a relationship can be discovered, we have to find out whether it may be used for accelerating the process of deposition of regenerants.

I. METHODS

The determination of the true values of the bioelectric potentials depends on the method by which they are obtained. In this respect, the input impedance of the measuring set-up is of great importance. This was not considered in many of the published investigations. Howink [43] used a two-stage and two-channel d.c. valve amplifier to the output of which the recording instrument was connected. From the results obtained the author draws important conclusions referring to theoretical problems of the electro-physiology of plants. However, the paper does not contain an analysis of the input stage of the amplifier where the grid currents of the input valve may cause a polarization of the electrodes, so distorting the effective values of the potentials. The same also applies to Hosemann's circuit [44]. The latter is based on the principle of converting the d.c. into a.c. which then is amplified and rectified before being fed to a cathode-ray tube. But in this circuit there is a direct galvanic connection between the tested object with the electronic valve forming the input element, the grid currents of which obviously also caused polarization of the electrodes.

In circuits for measuring the bioelectric potentials of plants the method of tapping-off and amplifying the small constant and slowly varying e.m.f.s and small voltages is of an overriding importance. Likewise, it is necessary to reduce to the lowest limit the energy absorbed from the tested object, in order to avoid voltage drops in the measuring circuit.

The measurement of the constant and slowly varying potentials of plant cells was carried out by means of an oscillograph of type EO-4. Into the input circuit of the oscillograph an electromechanical transducer was connected according to Khvedelidze's suggestion [4]. As an electromechanical transducer we used a polarized relay type RP-4 (Fig. 1).

The input of the oscillograph is provided with an *RC*-differentiating element. Its total impedance is

$$Z(j\omega) = \frac{1}{j\omega C} + R. \tag{1}$$

From the source at the input of the *RC*-element we get rectangular pulses of a duration t'. The frequency characteristic of these pulses is determined by the condition

$$F(j\omega) = \int_0^{t'} e^{-j\omega x}\, dx = \frac{1}{j\omega}(1 - e^{-j\omega t'}). \tag{2}$$

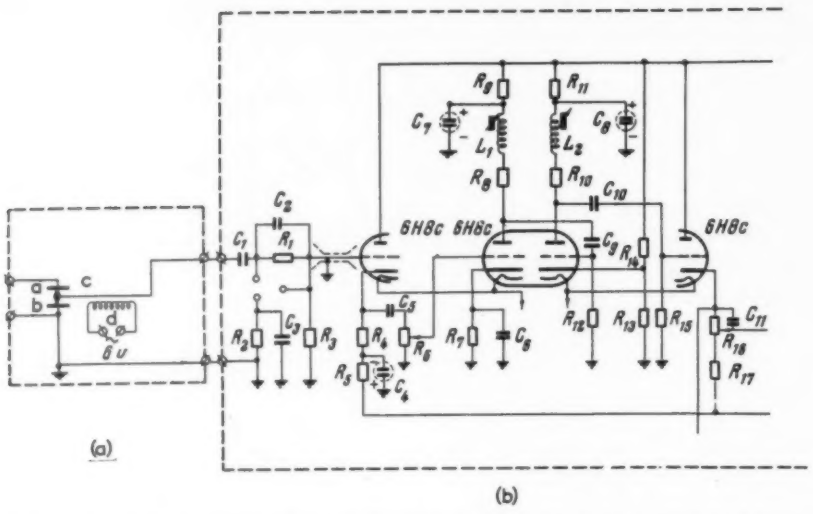

FIG. 1. Circuit diagram of input circuit of oscillograph EO-4 with mechanical modulator, used for measuring the bioelectric potentials of plant cells: (a)—mechanical amplitude transducer type RP-4; (b)—input of vertical amplifier of oscillograph type EO-4; a, b fixed, c moving contacts of relay; d armature supplied with a.c., voltage 6V;

C_1 = 0·25 pF±10 per cent, 600 V, leakage resistance not less than 10^{10} Ω
C_2 = 6·35 pF
C_3 = 200 pF±5 per cent, 500 V
C_4 = 20 μF, 150 V
C_5 = 4 μF±10 per cent, 200 V
C_6 = 4700 pF±10 per cent, 500 V
C_7 = 20 μF, 150 V
C_8 = 20 μF, 150 V
C_9 = 0·5 μF±10 per cent, 200 V
C_{10} = 0·5 μF±10 per cent, 200 V
C_{11} = 4700 pF±10 per cent, 500 V

R_1 = 2 MΩ±5 per cent, 0·5 W
R_2 = 240 kΩ±5 per cent, 0·5 W
R_3 = 2 MΩ±5 per cent, 0·5 W
R_4 = 100 kΩ±10 per cent, 1 W
R_5 = 220 kΩ±10 per cent, 1 W
R_6 = 100 kΩ±20 per cent
R_7 = 270 kΩ±10 per cent, 1 W
R_8 = 8·2 kΩ±10 per cent, 1 W
R_9 = 27 kΩ±10 per cent, 1 W
R_{10} = 8·2 kΩ±10 per cent, 1 W
R_{11} = 27 kΩ±10 per cent, 1 W
R_{12} = 1 kΩ±10 per cent, 1 W
R_{13} = 270 kΩ±10 per cent, 1 W
R_{14} = 56 kΩ±10 per cent, 1 W
R_{15} = 1 kΩ±10 per cent, 1 W
R_{16} = 22 kΩ±20 per cent
R_{17} = 270 kΩ±10 per cent, 1 W

The input circuit is a linear system in which there is direct proportionality between the magnitude of the input quantity and the magnitude of the variations caused by it in the system. To this circuit we apply the principle of superposition, according to which, firstly, the result of several simultaneous actions on the system considered is equal to the algebraic sum of the results obtained in the case where each action is applied separately and, secondly, individual frequency components, into which the e.m.f. of a constant pulse acting in the element may be resolved, are independent of each other. Therefore the determination of current and voltage in the input circuit of the oscillograph is possible by summing the elementary components of the effect of the e.m.f. applied to it. We then get:

$$i(t) = \frac{1}{2\pi} \int_{+\infty}^{+\infty} \frac{F(j\omega)}{Z(j\omega)} e^{j\omega t}\, d\omega = \frac{1}{R}\left[e^{-t/RC}\, 1(t) - e^{-(t-t')RC}\, 1(t-t')\right]. \quad (3)$$

Equation (3) shows that the range of negative voltages will be observed on the resistances of the circuit for $t > t'$ if after the end of the application of the pulse there lapses a certain interval of time, and $R_{\text{int.}}$ of the source of the e.m.f. is 0. However, in the RC-element there follows after each pulse a discharge of C_1 on $R_{\text{inp.}}$ during the same time t'. Also, since the switch-over of the moving contact of the relay RP-4 takes a certain time, the discharge of $C_{\text{inp.}}$ does not follow instantaneously. Practically the discharge current is equal to the charging current, viz.

$$i_C = C\frac{dE_x}{dt} = \frac{E_x}{R} e^{-t'/RC} = \frac{U_C}{R} e^{-t'/RC}. \quad (4)$$

It has been established [4, 49] that if at the output of the RC-element the relation $t_1/RC = 0{\cdot}02$ holds, the form of the rectangular pulse in the cathode-ray tube coincides with the form of the pulse at the input of the circuit.

In the circuit converting constant potentials into pulses the magnitude of the grid current of the input valve of the amplifier cannot have any effect on the results of the measurement of the e.m.f. of plant cells. An equivalent circuit in d.c. conditions of the differentiating input element may take the form of a divider of the voltage V, consisting of the active leakage resistance R of the capacitor and R in the grid circuit of the valve. Considering the high insulation resistance of the input capacitor ($>10^{10}$ ohm), the leakage current is extremely small and may be neglected [4]. Prior to the connection to the e.m.f. investigated, $C_{\text{inp.}}$ is charged to the value of the voltage drop produced in the resistance by the grid current. Before the measurement of the potentials the magnitudes of voltage in the parts of the RC-element of the vertical amplifier of the oscillograph are strictly equal, and at the input terminals the bioelectric potential = 0. In this way, penetration of the grid current into the plant cells during the measurement is prevented. The sensitivity of the oscillograph tested with a constant potential through an additional input circuit was 1 mV/mm. A sensitivity of this order is quite acceptable, since (1) the bioelectric potentials of plant cells have values of the order of some tenths of millivolt, so that the precision of the measurements in the first stages of the investigation of our problem need not be greater than $\pm 10^{-1}$ mV; (2) the circuit adopted assures an undistorted reproduction of the potentials investigated with absence of any polarization of the object; (3) the input element absorbs only a negligible electric charge from the measured e.m.f.

The input terminals of the fixed contacts of the relay were set into amber, to improve their insulation. The moving contact of the input circuit had also high-grade insulation. The clearance between the contacts was regulated by micrometric screw. This regulation is required to obtain equality of charging and discharging times of the input capacitor of the system.

The object investigated was carefully screened against external electric and electromagnetic fields. For this purpose we designed a screened chamber which was carefully earthed. Since there is a bioelectric potential of photosynthesis in plants,

actual measurements and control measurements were carried out in identical illumination.

To measure the bioelectric potentials we used silver silver-chloride electrodes Ag/AgCl. There should not be an inherent potential in the electrodes themselves. The absence of any inherent potential and of polarization phenomena was established from the indication of the beam of the cathode-ray tube when the electrodes were immersed in a KCl solution. It has been suggested [4, 45] to measure the inherent potential of the electrodes in NaCl solution. However, this procedure cannot be recommended. It does not consider the presence of a diffusion potential in the NaCl.

The silver silver-chloride electrodes were not applied direct to the object, to avoid the penetration of silver ions into it, etc. To connect the electrodes with the system investigated we used various forms of small tubes filled with tap water or pure agar with KCl. To the plant cells we applied tubes with agar either direct or small tufts of camel hair steeped in water. The whole system (electrode—intermediate link—tuft of hair—tuft of hair—intermediate link—electrode) was tested by indication of the beam of the cathode-ray tube of the oscillograph for the presence of any potential. If a small potential was found in the system, its absolute value and polarity was considered in the control and the actual experimental measurements. The distance between intermediate links was kept strictly constant in each test and control series. In the measurements of the bioelectric potentials great attention was paid to a good contact between the camel-hair tuft and the biological system. We were interested in the features of the bioelectric potentials immediately associated with the metabolic processes of the cell systems investigated. In the case of damaged cells there is always a new more or less appreciable injury potential. The part having suffered the trauma becomes electro-negative relatively to the normal surface. The "demarcation" potential observed is strong and sometimes completely distorts the metabolic bioelectric potential. We did not regard it as appropriate to use as intermediate elements between electrode and object a solution of NaCl, particularly in high concentrations [4]. The ions of salt surrounding the cells and penetrating into them alter the bioelectric potential of the system, and in high concentrations may produce plasmolysis. It is even possible for considerable diffusion potentials to be set up in the intermediate links. A number of authors [4, 9] lead off the bioelectric potentials of plant cells by means of platinum electrodes. This, again, is hardly appropriate, since the potential of these electrodes depends strongly on the conditions of the system under investigation. The potential of the platinum electrodes themselves varies considerably with the concentrations of oxidized and reduced compounds and of hydrogen ions.

2. VARIATIONS OF BIOELECTRIC POTENTIAL IN THE FORMATION OF THE CELLS OF A CALLUS AND OF SECONDARY MERISTEM

As objects of the investigation we used the tomatoes Shtambovii Alpat'eva No. 0905a, Gruntovyi Gribovskii No. 01180, and "Best of All" No. 318. The experiments were carried under greenhouse and field conditions, in the Botanical Gardens of Moscow University. To obtain the calluses on the stem of the tomato the top of each plant was cut off above a certain leaf. The calluses were obtained above leaves 1, 3, 5, 7, 9, on the peduncles and leaves. As a preliminary measure all lateral buds were removed from the tomatoes under investigation.

Prior to the experiment the plants, preliminarily planted out in separate pots, were assembled into groups of 10–15 plants. The tomatoes were so selected that the cells of the stem which after the operation were on the surface had approximately the same potential differences. The bioelectric potential of the cells of the stem and the callus of the same individual of a given series were measured at many points, i.e. all over the mass of the tissue investigated. The distance (spacing) between the intermediate links was 2–3 mm.

During the first days after the operation it was not possible to establish under our experimental conditions any definite regularity in the variation of the bioelectric potential at the point of the cut. The value of the potential depended on the character of the injury of the cells, the condition of the liquid on the cut, the drying of the scab that had formed, etc. Relative to the tissues above and below it the point of the cut was characterized by electro-negative polarity. With progressive formation of the scab, covering the whole surface of the cut on the stem, the polarity often changed to take a positive value.

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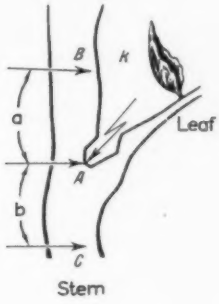

FIG. 2. Diagrams of application of electrodes: *k* point of appearance of callus; *a*, *b* stages of development.

FIG. 3. Diagrams of application of electrodes: *k* point of appearance of callus; *a*, *b* stages of development.

With incipient formation of the callus the variation of the bioelectric potential became more regular. When its rudiments appear, the bioelectric potentials rise slightly. The difference between the bioelectric potential of the original parenchymatous cells of the stem and the bioelectric potential of the parenchymatous cells of the callus in the initial stages of its formation reaches 15·5–19 mV for Shtambovii Alpat'eva and 11·0–15·0 mV for "Best of All". Later on the bioelectric potentials continue to rise (Table 1). However, later, before the emergence of the rudiment of the meristem, the bioelectric potential decreases compared with the preceding stage, by 31–38 mV in Shtambovii Alpat'eva and to 19–22 mV in "Best of All". The stage

of the callus, characterized by a reduced value of the bioelectric potential, does not last long. Directly accessory growing points are formed there is a rise again of the bioelectric potentials by 21–28 mV in "Best of All" and by 24–27 mV in Shtambovii Alpat'eva. The variation of the bioelectric potential in the process of callus formation does not depend either on its dimensions or on its location on the stem of the mother plant (Table 1).

TABLE 1. CHANGE OF BIOELECTRIC POTENTIAL DIFFERENCE (mV) DURING REGENERATION OF TOMATOES

Stages of growth	Shtambovii Alpat'eva					"Best of All"				
	Absolute value of bioelectric potential	Relative change of bioelectric potential compared with preceding stage				Absolute value of bioelectric potential	Relative change of bioelectric potential compared with preceding stage			
Calluses above third leaf of mother plant										
Stem	18·0	—	—	—	—	7·0	—	—	—	—
Callus	37·0	+19·0	—	—	—	18·0	+11·0	—	—	—
	52·0	+34·0	+15·0	—	—	26·0	+19·0	+8·0	—	—
	14·0	−4·0	−23·0	−38·0	—	4·0	−3·0	−14·0	−22·0	—
Growing point	42·0	+24·0	+5·0	−10·0	+28·0	31·0	+24·0	+13·0	+5·0	+27·0
Calluses above fifth leaf of mother plant										
Stem	21·5	—	—	—	—	5·5	—	—	—	—
Callus	38·0	+16·5	—	—	—	19·0	+13·5	—	—	—
	55·0	+34·0	+17·5	—	—	24·0	+18·5	+5·0	—	—
	17·5	−4·0	−20·5	−38·0	—	5·0	−0·5	−14·0	−19·0	—
Growing point	47·5	+26·0	+9·5	−8·0	+30·0	29·5	+24·0	+10·5	+5·5	−24·5
Calluses above seventh leaf of mother plant										
Stem	22·0	—	—	—	—	6·5	—	—	—	—
Callus	37·5	+15·5	—	—	—	21·5	+15·0	—	—	—
	53·5	+31·5	+16·0	—	—	27·0	+20·5	+5·5	—	—
	22·5	+0·5	−15·0	−31·0	—	5·0	−1·5	−16·5	−22·0	—
Growing point	44·0	+22·0	+6·5	+21·5	+21·5	30·0	+23·5	+8·5	+3·0	+25·0

NOTE. The results were obtained by measurements carried out every 10 days during the experiment.

The data on Eh, rH_2, pH_n of the activity of some enzymes (catalase peroxidase, polyphenoloxidase, ascorbicoxidase) and respiration of the cells, which we obtained on the same objects, reveal a far-reaching parallelism between their variations and the character of the behaviour of the bioelectric cell potentials. The initial stages of the formation of the callus and of accessory growing points with high values of the bioelectric potential were invariably characterized by increased physiological activity. During these periods the growth of the cell structures proceeded at the highest rate.

Table 2 shows the data on the variation of the electropolarity and of the values of the potentials during the development of the calluses at points from which axillary buds have been removed. In the different organisms the values of the bioelectric potential and signs of the polarity relatively to tissues situated above and below the callus varied considerably. In addition, the results obtained on one and the same object also exhibited great variations of the magnitudes of the bioelectric potential, according to the stage and conditions of growth of the callus. Nevertheless, the facts

TABLE 2. CHANGE OF ELECTROPOLARITY AND MAGNITUDE OF THE BIOELECTRIC POTENTIAL (mV) DURING FORMATION AND DEVELOPMENT OF THE CALLUS AT THE PLACE OF REMOVAL OF AN AXILLARY BUD ON THE TOMATO SHTAMBOVII ALPAT'EVA

Stage of growth	Wound Surface		Callus			Rudiments of Growing Point			
	Days after Operation								
	10	20	31	40	50	57	70	80	85
a	−25	+17	+(13–20)	+(10–17)	−10	+(10–23)	−(8–17)	−15	+17
b	−30	−11	+(16–20)	−(15–25)	+13	+8	−(10–21)	+20	−22

NOTE. Before the experiments the tops of the stems and all buds were removed from the plants. The sign of the bioelectric potential is taken relative to point A (Fig. 2).

established enable us to draw the conclusion that (1) in every case the rudimentary calluses have a positive potential relative to cells above and below them; (2) growing points in formation are also characterized by electro-positive polarity; (3) the magnitude of the bioelectric potential increases in both cases by comparison with preceding stages.

Table 3 shows a characteristic case of a variation of the bioelectric potential during the formation of the callus and of accessory growing points where the top of a stem had been removed. Here, again, it was invariably observed that the rudiments of young calluses and of accessory growing points are characterized by electro-positive polarity and an increase of the bioelectric potential.

TABLE 3. CHANGE OF THE ELECTROPOLARITY AND MAGNITUDE OF THE BIOELECTRIC POTENTIALS (mV) ON FORMATION OF CALLUS AND ON ACCESSORY GROWING POINT AT PLACE OF REMOVAL OF TOP OF STALK OF THE TOMATO "BEST OF ALL"

Stage of Development	Wound Surface			
	Days after operation			
	10	20	30	
a	−(15·5–16·5)	+(8–10)	+(16–16·5)	−(13·5–15)
b	−(14–14·5)	+(20–22)	+(12·5–13·5)	−(8·5–10)

Stage of Development	Callus						Growing Point	
	Days after operation							
	35	50	60		70		73	90
a	+(21–21·5)	+31·5	+21·5	−(23·5–25·5)	−(5–13)	+15	+27·5	−23·0
b	+(14–15)	+23	−(13·5–14)	−(16·5–20·5)	+(26–28)	−32	+58	+37·5

NOTE. The sign of the polarity of bioelectric potential Rp taken relative to point A (Fig. 3). In the table the most characteristic case of change of electropolarity during the initial stages of the regeneration is reproduced.

The measurements of the bioelectric potentials of the calluses and growing points were carried out daily, and at the same time of the day, under strictly defined conditions. In each test series 25 plants were tested.

3. EFFECT OF THE BIOELECTRIC POTENTIAL ON THE REGENERATION OF THE PLANTS

To analyse the interrelations of the bioelectric e.m.f.s with physiological processes, and to clarify the nature of the biopotentials, we require some data on the character of the effects of electricity on the various tissues.

As stated above, a potential positive relatively to that of the tissues above and below it is set up at the point of the cut on the stem as soon as a callus forms. If at this time the positive electrode of a d.c. source of 1 V is applied to it, the negative electrode being placed at the bottom of the stem, the intensity of respiration of the cells of the callus changes. The electrodes were so placed that any traumatic effect was absolutely excluded, because wounds have also a considerable influence on the respiration and bioelectric potential of the cells [2, 46]. The current passing through the tissues of the stem was of the order 0·0018 mA. Fig. 4 is a set of curves each of which

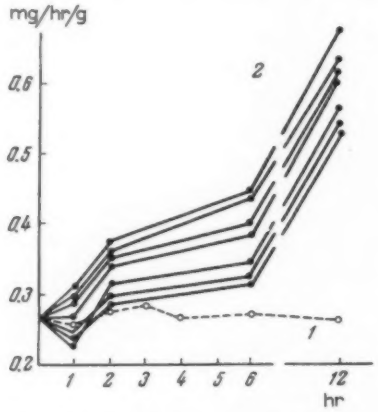

FIG. 4. Changes of intensity of respiration of cells of the stalk of tomato "Best of All" under influence of an additional potential (voltage 1 V, current 0·0018 mA). §1 control; 2 test. Abscissa; duration of application of e.m.f. to tissues of stem; ordinate CO^2 mg/hr/per g of moist substance.

is the mean value of four plants. Measurements of the intensity of respiration were carried out after the termination of each test, i.e. after 1, 2, 6, and 12 hr, respectively. In all cases the intensity of respiration increased in the zone of the positive potential. Exceptions were observed only in some cases when the potential was applied for only 1 hr. It was not possible to establish the cause of this; it is possible that in such a case the additional potential has an adverse effect, but it cannot be excluded either that the intensity of respiration of the cells of these plants may have been considerably lower than the mean control value prior to the test. In all other cases the intensity of the respiration increased with increasing action of the extraneous e.m.f. With a 2-hourly dose it increased by 7–12 per cent, with 6-hourly dose by 26–35 per cent, and with a 12-hourly dose by a factor 2–2·5.

The length of the stem between the electrodes was 25 cm.

When the potential of an extraneous e.m.f. is applied, the pH_n of the plant cells varies. As the data given in Fig. 4 show, the activity of the hydrogen ions in the tissues of the stem near the positive electrode rose, although not significantly. In the control the pH_n of the cells was 5·67, in the test 5·55–5·40. When the additional potential applied to the cells was of a low voltage and the current weak (1·2 V and 0·002 mA) and the time of application limited, the activity of the hydrogen ions after the test returned rapidly to the initial value, i.e. pH_n increased (Fig. 5 and Table 4).

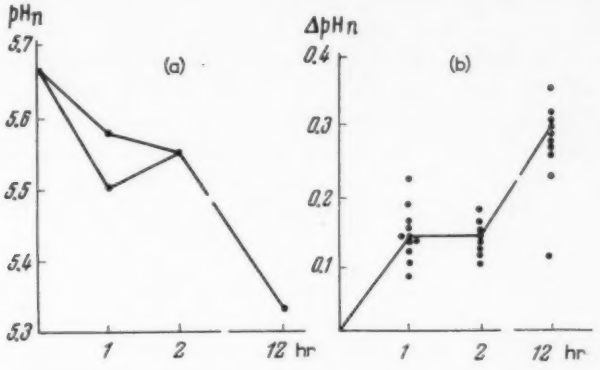

FIG. 5. Changes of pH of the cells of the stem of tomato "Best of All" on application of additional potential to them (voltage 1 V, current 0·0018 mA). §Abscissa of both graphs: duration of application of e.m.f. to tissues of stem; (a) measurements carried out directly after each test; (b) measurements carried out 24 hr after each test.

TABLE 4. EFFECT OF AN ADDITIONAL POTENTIAL ON THE VALUES OF Eh, rH_2, AND pH_n OF THE CELLS OF THE STEM OF THE TOMATO SHTAMBOVII ALPAT'EVA

Duration of action, hr	Eh (V)	pH_n	rH_2
Measurements carried out directly after each test			
Control	+0·2117	5·67	18·64
1	+0·2899	5·54	21·07
2	+0·2960	5·55	21·31
12	+0·2895	5·40	20·77
Measurements carried out 24 hr after each test			
Control	+0·2355	5·66	19·44
1	+0·2030	5·70	18·40
	+0·2712	5·64	20·63
2	+0·2497	5·81	20·23
	+0·2588	5·80	20·52
6	+0·2315	5·98	19·94
	+0·2926	5·48	20·70
12	+0·2441	5·95	20·32
	+0·2706	5·69	20·71

When an additional potential is applied to the tissues of the stem of a tomato, taking into account their natural electropolarity, a considerable change of the oxidation-reduction potential is observed. In a case in which the Eh of the callus cells was +0·2117 V, the application of the positive electrode connected with an element of 1·2 V and yielding 0·001 mA resulted within 1 hr in an increase of the oxidation-reduction potential to +0·2899 V, and within 2 hr to +0·2960 V. When an extraneous e.m.f. is applied to the callus cells, the rH_2 increases within 1 hr by 2·43, and within 2 hr by 2·67 units. The electrode connected to the negative pole of the battery was applied to the base of the stem. The height of the plant was 32 cm.

The difference in the Eh and rH_2 values for the experimental plants has a more stable character than the variation in the pH_n. The increase of the values of Eh and rH_2 is maintained for at least one day after the application of the potential (Table 4).

Under the effect of an extraneous e.m.f. not only does the character of the physico-chemical parameters of the state of the cells vary, but the cyto-physiological processes themselves change. We determined the reducing power of the cells by Hatry's method. It was found that with small doses of the extraneous e.m.f. the reducing activity of the callus cells increases considerably (Fig. 6a). With increasing dose it subsides, but later, when the potential is applied to the tissues of the stem for 12 hr, the reducing power exceeds the values of the control plants. The same applies also to the content of ascorbic acid of the callus cells (Fig. 6b). When a positive potential is applied to them for 1 hr the content of ascorbic acid increases by 15 per cent. However, with a 2-hr dosage the content drops by 45 per cent. Later, with an increase of the duration of the applied potential, the concentration of ascorbic acid in the cells near the anode rises again.

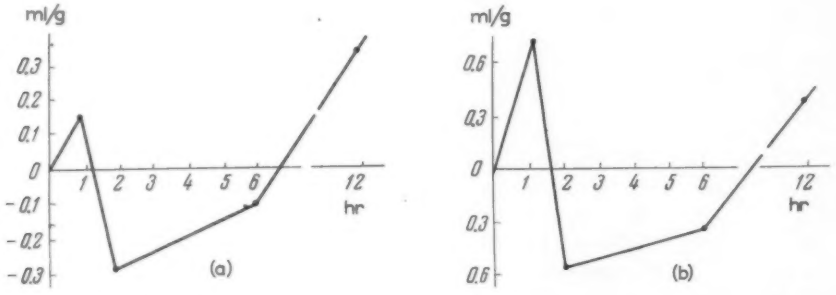

FIG. 6. Changes of content of ascorbic acid (a) and of the reducing power of the cells (b) on the application of an additional potential (voltage 1·2 V and current 0·002 mA) to the tissues of the stem of the tomato "Best of All". In all cases the data of the control test were taken as zero. Abscissa of both graphs indicates duration of application of the extraneous e.m.f. to tissues of the stem, ordinate (Fig. 6a) 0·001 N 2: 6-dichlorophenolindophenol, ml/g of moist substance; ordinate (Fig. 6b) 0·005 N iodine ml/g of moist substance.

The accessory growing points on the callus of the tomato were formed while there was increased activity of the hydrogen ions in the tissues and the Eh, rH_2, and bioelectric potential had high values. The pH_n of these cells was about 5·1 for "Best of All" and 5·4 for Shtambovii Alpat'eva. The pH_n values of the original cells of the stems were 6·5 and 6·6, respectively. For the growing points of the regenerants Eh

was +164 mV for Shtambovii Alpat'eva and +53 mV for "Best of All", whereas Eh for the cells of the stems was only +35 mV and −81 mV, respectively. Lastly, if the cells of the growing points at the beginning of their differentiation had a rH_2 equal to 12·9 for "Best of All" and 16·8 for Shtambovii Alpat'eva, the cells of the stem were characterized by lower values (18·1 and 12·1). Directly after formation of the regenerants in the callus cells, an increase of reducing power and maximum content of ascorbic acid were observed. During the period at which accessory growing points appeared, as stated above, there arises a steady positive electropolarity of these points themselves relative to the tissues of the stem underneath as well as to the cells of the leaf petiole.

On the other hand, given the appropriate experimental conditions, it is possible to produce the above-mentioned characteristics of the condition of the intra-cellular physiological processes artificially by an application to the cells of an additional potential at optimum dosage and taking into account the natural electropolarity of the tissues.

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The facts stated above suggested that it might be possible to accelerate the regeneration process of tomatoes by applying a potential to the tissues of the stem.

TABLE 5. ACCUMULATION OF TOTAL NITROGEN IN VARIOUS PARTS OF TOMATO PLANTS "BEST OF ALL" ON IMMERSION OF THE STEM IN AN AQUEOUS SOLUTION OF 0·5 PER CENT ASPARAGINE AND 0·5 PER CENT GLYCINE AND APPLICATION TO IT OF AN ELECTRIC POTENTIAL (VOLTAGE 1 V AND CURRENT 0·0018 mA). HEIGHT OF PLANTS 24 cm; DURATION OF TEST 6 hr

Total nitrogen	Level in plant (cm)	Nitrogen content per absolute dry weight (per cent)	Exptl. variants in reln. to 1st Control	Gain of nitrogen from treatment with electricity (corresponding levels of 2nd Control taken as 0 per cent)
1 Control				
Stem	0–6	0·99	—	—
	6–12	1·08	—	—
	12–18	1·14	—	—
	18–24	1·28	—	—
2 Control	Series A			
Stem	0–6	1·02	0·03	—
	6–12	1·11	0·03	—
	12–18	1·25	0·11	—
	18–24	1·27	0	—
	Series B			
Stem	0–6	1·30	0·31	—
	6–12	1·66	0·58	—
	12–18	1·44	0·30	—
	18–24	1·52	0·24	—
Test	Series A			
Stem	0–6	2·14	1·15	1·12
	6–12	2·07	0·99	0·96
	12–18	2·18	1·04	0·93
	18–24	2·22	0·94	0·94
	Series B			
Stem	0–6	2·25	1·26	0·95
	6–12	2·33	1·25	0·67
	12–18	2·46	1·32	1·02
	18–24	2·59	1·31	1·07

TABLE 6. ACCUMULATION OF PROTEIN NITROGEN IN VARIOUS PARTS OF TOMATO PLANTS "BEST OF ALL" WHEN THEIR STEMS ARE IMMERSED IN AN AQUEOUS SOLUTION OF 0·5 PER CENT ASPARAGINE AND 0·5 PER CENT GLYCINE AND AN ELECTRIC POTENTIAL IS APPLIED TO THEM (VOLTAGE 1 V AND CURRENT 0·0018 mA). HEIGHT OF PLANTS 24 cm; DURATION OE TEST 6 hr.

Protein nitrogen	Height in plant (cm)	Nitrogen Content		Exptl. variants in reln. to 1st control	Gain of nitrogen from treatment with electricity (corresponding levels of 2nd control taken as 0 per cent)
		In per cent of absolute dry weight	In per cent of total nitrogen		
1 Control					
Stem	0–6	0·46	46·5	—	—
	6–12	0·61	56·5	—	—
	12–18	0·72	63·2	—	—
	18–24	0·84	66·0	—	—
2 Control					
Stem	Series A				
	0–6	0·79	77·5	0·33	—
	6–12	0·82	73·9	0·21	—
	12–18	0·87	69·6	0·15	—
	18–24	0·88	69·3	0·04	—
Stem	Series B				
	0–6	1·06	81·5	0·60	—
	6–12	1·35	81·3	0·74	—
	12–18	1·12	77·8	0·40	—
	18–24	0·99	65·1	0·15	—
Test	Series A				
Stem	0–6	1·24	57·9	0·78	0·45
	6–12	1·19	57·5	0·58	0·37
	12–18	1·49	68·3	0·77	0·62
	18–24	1·58	71·1	0·74	0·70
	Series B				
Stem	0–6	1·37	60·9	0·91	0·31
	6–12	1·35	57·9	0·74	0
	12–18	1·74	70·7	1·02	0·62
	18–24	1·96	75·7	1·12	0·97

This assumption became more real when the results of the following experiments were available. In a selected group of "Best of All" tomato plants 24 cm in height the tops of the stalks were removed, as well as all the axillary buds, at the height of the fifth leaf. Towards the tenth day after the operation the points of the cut assumed a steady positive potential relative to the petiole of the leaf as also to the cells of the stalk. On the eleventh day the plants were cut and placed in an aqueous solution containing 0·5 per cent asparagine and 0·5 per cent glycine.

A thin layer of moist cotton wool was put on the experimental plants at the points where the tops of the stalks had been cut and where the accessory buds of the regenerants should form, and this was connected to the positive electrode of a battery of 1 V. The top of the electrode was also covered with cotton wool. The negative electrode was immersed in the vessel with the solutions of amino-acids. The same operation was carried out on the control plants, except that the leads were not connected to a battery.

All the plants of one series, viz. 10 experimental and 10 control plants, were placed

in one vessel. The experiment lasted 6 hr. The current supplied by the external e.m.f. to the stalk was 0·0028 mA.

An analysis of the total nitrogen content and the protein nitrogen indicated great changes in the synthetic processes of the cells as a result of the external artificially applied potential. Of the two figures of the control entered in Tables 5 and 6, one indicates the level of nitrogen under ordinary conditions of growth of the tomatoes, and the second the nitrogen level in the case where the stalks were cut and placed in a vessel with amino-acids.

The maximum gain in total nitrogen in the experimental as well as control plants was observed on cells at heights of 6–18 cm. However, in the control plants at a level of 18–24 cm the nitrogen gain was 0–15·8 per cent, but in the experimental plants it reached 42·3–50·6 per cent. In experimental plants the gain in total nitrogen content in the whole stalk was considerably greater than in the control plants. For example, at the various zones of height this gain, as a percentage of the total nitrogen content, was:

Height of Plant (cm)	Second Control (Per cent)	Experiment (Per cent)	
		A	*B*
0–6	23·8	51·4	56·0
6–12	34·9	47·8	53·6
12–18	20·8	47·7	53·7
18–24	15·8	42·3	50·6

From the application of the external potential in series *A*, by height zones, the gain in total nitrogen content as a percentage of the latter was 52·3; 46·3; 42·7; 42·3; and in series *B*: 42·2; 28·8; 41·5; 41·3.

Analogous data were obtained in an analysis of the protein nitrogen. The maximum gain in protein nitrogen in the test and in the second control was observed in cells of the base of the stalk, i.e. the part immersed directly in the solution. The higher the zone, the smaller the nitrogen content in the control plants. The character of the percentage ratio of the gain of protein nitrogen to the total nitrogen content according to height zones in the experimental plants was slightly different:

Height of Plant (cm)	Second Control (Per cent)		Test (Per cent)	
	A	*B*	*A*	*B*
0–6	41·8	56·6	62·9	66·4
6–12	25·6	54·8	48·7	54·8
12–18	17·2	35·7	51·7	58·6
18–24	4·5	15·2	46·8	57·1

Not taking account of the part of the stalk immersed in the asparagine-glycine solution, the greatest gain in protein nitrogen was observed just in the tissues near the positive potential, i.e. at the levels 12–24 cm. The direct gain in protein nitrogen from the extraneous e.m.f. as a percentage of the total nitrogen content in series *A*

was, according to height zones: 36·3; 31·1; 41·6; 44·3; and in series *B*: 22·6; 0; 35·6; 49·5.

Therefore, if in the stalk cells located about the positive potential the increase in *total* nitrogen content due to the additional potential was slightly less than in the lower zones underneath, yet the *protein* nitrogen was greater just in these tissues. Comparing the contents of total and protein nitrogen with the content of dry substance in the tissues of the control and experimental plants puts the influence of the additional potential transport and synthesis beyond doubt. This conclusion is also drawn from an analysis of the nitrogen content in the tomato stalks when the concentration gradient of the individual zones is considered. Taking the total nitrogen content at the base of the stalk as 0 per cent, we get:

Height of Plant (cm)	Control 1 (Per cent)	Control 2 (Per cent)		Experiment (Per cent)	
		A	*B*	*A*	*B*
0–6	0	0	0	0	0
6–12	0·09	0·09	0·36	−0·07	0·08
12–18	0·15	0·23	0·14	0·04	0·21
18–24	0·29	0·25	0·22	0·08	0·34

Correspondingly for the albumen nitrogen:

Height of Plant (cm)	Control 1 (Per cent)	Control 2 (Per cent)		Test (Per cent)	
		A	*B*	*A*	*B*
0–6	0	0	0	0	0
6–12	0·15	0·03	0·29	−0·05	−0·02
12–18	0·26	0·08	0·06	0·25	0·37
18–24	0·38	−0·09	0·04	0·34	0·59

The results on gain of nitrogen by levels of the stalk show, firstly, that during the experiment the gradient of the nitrogen concentration in the second control is smoothed or even changes its character since the maximum of its gain does not reach the top levels of the stalks and, secondly, the gradient of the concentration is considerably intensified in the experimental plants, and particularly in the cells situated near the positive potential, i.e. at the highest levels of the stalk.

When a potential is applied to plant cells the gradients of the concentrations of the ions of various substances change considerably. Much experimental material has been accumulated that shows that extraneous e.m.f.s have without any doubt an influence on the physiological systems of the cells by altering their ion concentrations [2, 25]. Since, according to the second law of thermodynamics, it is necessary for a system to have an energy gradient in order that it may perform work, the potential may, most probably, play a definite role in the transport of substances through the tissues. The capacity acquired by higher plants of transporting nutrients is influenced by a number of factors regulating its speed and direction. The transfer of substances is a complex physiological process indissolubly associated with the metabolism [47].

Plastic substances migrate in the direction of increasing capacity of the protoplasm for adsorptive saturation with these substances, i.e. in the direction of the gradient of adsorption [22, 48, 49]. The mechanism of the migration of substances is, according to Kursanov [50], similar to the active function of the protoplasm in determining the penetration of ions into plant cells. If that is so, the transport of substances must be associated with an increase in the metabolic activity of the cells, expressed, particularly, by an intensification of respiration. According to the work of Kholodnyi, Went, Katunskii, and others [5, 19, 20, 23], the growth substances of the plants carry an electric charge and are moved cataphoretically under the influence of the bioelectric potential.

The electric potentials may therefore influence the transport of substances in two different ways: firstly, directly by the electropolarity of the tissues; secondly, by intensification of cell respiration.

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Concluding, we report the results of yet another experiment in which an additional potential was applied to the sites of callus formation. The experiment was carried out according to the scheme described, but the plants were not cut and the treatment was continued for five days. The actual duration of the passage through the plant tissues was obviously shorter because during the night hours the cotton wool dried, and the contact between battery and plant was interrupted. The tomatoes were grown in pots in the greenhouse. All the individuals had calluses that were at the stage at which the formation of the growing points of the regenerants was expected. Fig. 7

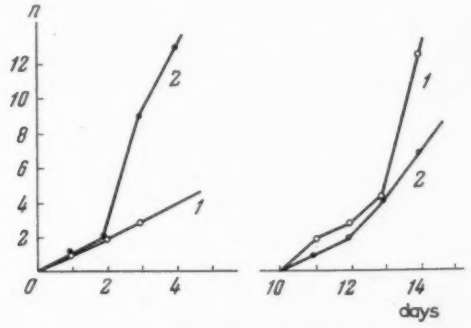

FIG. 7. Formation of regenerants on calluses of tomato "Best of All" on application of an additional potential to them (voltage 1 V, current 0·0028 mA). 1 control; 2 test. Abscissa: days from application of external e.m.f. to tissues of callus of tomato; ordinate: number of calluses with regenerants.

shows that during the first days after the connection of an external e.m.f. to the tomatoes, accessory buds of the regenerants on the calluses formed much more rapidly on the experimental plants. Only around the tenth day the daily rate of the formation of growth points on the control plants became equal to that on the experimental plants, and at the time at which the stage of maximum laying down of the regenerants was at an end in the experimental plants, it was only just beginning in the control plants. In the best variants the regeneration process was accelerated by a factor of three times. Where negative potentials were applied to the calluses, the formation of additional growth points was considerably delayed. The control plants outstripped the experimental plants in this case by two to three weeks.

In the cases in which the voltage or the dose of the potential applied were large, a dissolution of the cells was noticed near the negative electrode. Cavities formed inside the stalk, at the surface of the tissues appeared "burns", whether the potentials of the external e.m.f. were positive or negative. The cells died in these regions and under these conditions there was no regeneration process.

Therefore: (1) since the bioelectric potentials of plant cells have very small absolute values (i.e. of the order of a few mV), positive results on variations of the morpho-physiological processes by the application of additional potentials "are obtained where low, but constant voltages are used" [34]; (2) to obtain positive results it is also necessary to preserve the natural polarity of the individual organs and of the whole organism.

CONCLUSIONS

(1) The present communication reports results of investigations of variations of the bioelectric potentials during the regeneration process in plants.

(2) We established definite variations of bioelectric potential in the cells of a callus: the initial stages of its formation are characterized by higher bioelectric potentials; in the stage preceding the formation of the rudiments of the secondary meristem the bioelectric potential decreases. The inception of the formation of accessory growing points is again characterized by a high potential.

(3) Young calluses and growing points are as a rule characterized during their formation by positive electropolarity.

(4) The regeneration process in tomatoes proceeds only in the case where, during the deposition of the calluses and differentiation of the growing points, the oxidation-reduction and bioelectric potentials have high positive values, and during the formation of the secondary meristem, the minimal positive, or actually negative, values.

(5) The bioelectric potentials are not mere products of metabolism, i.e. its "waste", but play an active role in the transport of substances and in the vital functions of the cells, the growth of which they activate.

(6) When an external e.m.f. is applied to the cells of the stem of a tomato, with due choice of the optimum dose and consideration of the natural electropolarity, the regeneration process is accelerated. The regeneration is inhibited if a potential with inverse polarity or of too high a voltage or current is applied to the zones of tissue growth.

(7) Attention should be drawn to the possibility of using small electric currents at small constant voltage for the acceleration of formative processes in plants.

Translated by B. F. KRAUS

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ON CHANGES IN OXYGEN UPTAKE OF BRAIN TISSUE AFTER X-IRRADIATION

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(*Received* 1 *August* 1956)

UNTIL recently it was believed that the enzyme system of the respiratory cycle and tissue respiration in general were relatively unaffected by ionizing radiation. High radiation doses were required to achieve a substantial divergence from normal. Lately, however, work has appeared that shows that tissues are affected more powerfully by ionizing radiation than was previously supposed, and with doses of a tenth the strength.

Barron, Wolkowitz, and Muntz [1] have shown that respiration in slices of various tissues is markedly reduced for 4 hr after exposure to 200 r. Sillivan [2] observed a reduction of respiration for 24 hr in slices of the spleen of rats that had received a total body dose of 200 r. Richmond [3] reported a decreased respiration in rats after a total body dose of 800 r. Van Bekkum [4, 5] showed that rat spleen and thymus mitochondria have a reduced uptake of oxygen for 2 hr after the animals have been exposed to 1100 r or even 300 r total body irradiation.

Manoilov [6] observed a depressing effect of radiation on tissue respiration of the isolated heart. He believes that the radiant energy depresses the aerobic phase of respiration.

Mytareva [7] studied tissue respiration and coupled oxidative phosphorylation in rabbits after doses of 1300 r and found substantial disturbances of the respiratory cycle, specific for the different tissues (spleen, liver, kidney, heart, muscle). Voskoboinikov [8] found that the activity of catalase, which takes part in biological oxidations, changes differently in different organs after irradiation.

It should also be noted that particularly in the early phases of the radiation syndrome blood circulation is affected, which might result in an inadequate supply of the tissues with oxygen. This has been studied extensively in experimental animals (rat, rabbit, and monkey) and in humans after radiotherapy [9–12].

The data in the literature, and our own observations on the changes of blood circulation produced by irradiation, do not give a quantitative picture of the oxygen balance of the tissues. If this were known one could assess the development of an appreciable oxygen lack during a severe fall of blood pressure, which might entail a disturbance of the functions of those organs that are especially sensitive to lack of oxygen, e.g. the brain.

In order to study to what extent the deficit of oxygen is a result of changes in the blood circulation on the one hand, or of changes in actual tissue respiration on the other, we have undertaken the investigations reported in this paper.

We used the polarographic method [13–15] to measure the concentration of free

oxygen in the brain. This method had been modified in our laboratory to suit the conditions of a chronic experiment. We have described this method in detail [16]; we would like, therefore, only to point out that, simultaneously with the estimation of the free oxygen content, for chronic experiments the use of what we have termed the "oxygen test" is extremely demonstrative and convenient for obtaining comparative results. The "oxygen test" consists of giving the experimental animal for an accurately defined time a known gas mixture containing an increased amount of oxygen.

In the case of an oxygen deficit and rapidly working oxidative processes this increased oxygen supply is utilized rapidly. In the case of an excess of oxygen or reduced respiration rate, the added oxygen will, on the other hand, result in an abnormally high oxygen level.

We inserted a platinum electrode 1–2 mm into the motor area of the cerebral cortex of the brain of a rabbit: 8–10 days after the operation, the animal was used for the experiment. The non-polarizable electrode was fastened to the skull on the day of the experiment. The oxygen concentration was estimated polarographically by measuring the diffusion current at 0·6 V. The changes in current were measured using a mirror galvanometer and recorded on a photokymograph. Simultaneously with the measurement of the free oxygen we measured the blood pressure, using the bloodless method of Busigin, Nefedov, and Levinskii [17].

We carried out three series of experiments with six rabbits in each. In the first series the animals received 1000 r and 1100 r whole-body irradiation. In the second series the heads only of the animals were given 3000 r and 1100 r. In the third series 2500 r and 1100 r were given to the abdominal region between the lower half of the thoracic vertebrae and the upper half of the lumbar vertebrae.

TABLE 1. CHANGES IN OXYGEN TENSION IN THE BRAIN DURING THE "OXYGEN TEST" IN IRRADIATED ANIMALS EXPRESSED AS PERCENTAGE OF INITIAL VALUE

No. of rabbit	Radiation dose (r)	Area irradiated	After							
			5–10 min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
27*	1100	Head	60	140	130	151	243	157	140	170
30	1100	Head	88	100	125	133	100	105	100	110
29	1100	Head	110	250	170	90	80	80	80	50
10	1150	Head	140	170	170	200	80	—	75	—
12	3000	Head	300	—	700	—	500	—	150	100
20	3000	Head	80	110	120	95	85	82	—	100
7	900	Whole body	390	547	323	382	340	500	—	200
2	1000	Whole body	86	143	107	94	94	104	107	—
5	1100	Whole body	184	—	150	—	145	—	71	—
13	1500	Whole body	50	130	100	—	70	—	78	—
18	1100	Whole body	140	110	80	Died of shock				
24	1100	Whole body	130	150	60	43	—	60	—	—

* Rabbit irradiated for a second time 30 days after first exposure.

The irradiation was carried out with a therapy tube run at 170 kV, 6 mA with 0·5 mm Cu and 0·75 mm Al filters. The dose rate was 40 r/min. We used male chinchilla rabbits of 2800–3500 g weight.

In the first and second series, practically immediately after the whole-body or head exposure, we observed an increase in oxygen concentration in the brain tissue which was quite considerable at times. In half of the experimental animals this happened about 5–10 min after the exposure. In the rest of the animals this phase was preceded by a short-lived depression of oxygen tension, which changed rapidly to an increase. In Table 1 are shown the changes in oxygen tension in the oxygen test in the rabbit brain expressed as percentage of the normal value.

One hour after the exposure the increase in oxygen tension became apparent in all cases. In most cases the oxygen tension increased for 2–3 hr after irradiation. About 4 hr after irradiation the picture changed, and in the majority of the animals the oxygen tension fell somewhat, later reaching the normal value or even less.

In Figs. 1 and 2 we give the photograms of the oxygen tests in rabbits Nos. 29 and 7, showing the change in oxygen tension during the first day after the exposure.

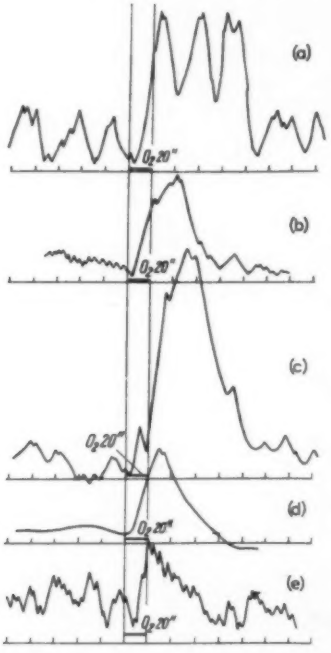

FIG. 1. Change of the diffusion current during the "oxygen test" before and after whole-body exposure. Rabbit No. 7. Signal = oxygen administration. (a) normal; (b) 7 min after exposure; (c) 1 hr after; (d) 3 hr after; (e) 8 hr after.

Thus, in the course of the first 2–3 hr after whole-body or head exposure there appeared a distinct maximum oxygen tension in the brain. In some of the animals (Nos. 7, 27, 30) this maximum remained for 5–7 hr and seemed to give rise to a second wave after a short depression. It should be noted that the time of onset of these maxima varies considerably from animal to animal, which reflects the individual differences in the development of the pathological process. The absolute magnitude of the changes of oxygen tension also varied considerably, and did not seem to depend

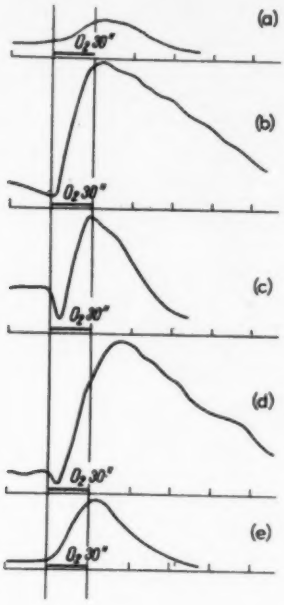

FIG. 2. Changes of the diffusion current during the "oxygen test" before and after whole-body exposure. Rabbit No. 29. Signal = oxygen administration. (a) normal; (b) 1 hr after exposure; (c) 2 hr after; (d) 5 hr after; (e) 7 hr after.

on the doses given. For instance, 900 r total-body exposure given to rabbit No. 7 resulted in great changes of the oxygen tension, whereas 3000 r given to the head of animal No. 20 gave only insignificant changes of the oxygen tension.

During the days after the exposure we observed a periodic change of the oxygen tension in the brain, which lasted throughout the time of the radiation disease, and up to the death of the rabbit. As a rule, the oxygen tension was increased on days 1 and 2. On days 2 and 3 there was a decrease of oxygen tension to normal and below, with a subsequent increase again on days 5–7.

In the third series in which the abdominal region was irradiated, the effect was of a different character. Five to 10 min after the end of the exposure, we regularly observed an oxygen tension about 20–40 per cent lower than the pre-irradiation level. This depression persisted and sometimes continued to fall, reaching its minimal value (rabbits Nos. 4 and 17) about 2–3 hr after irradiation. After this time, as a rule, there was some return towards normal (Table 2). The photogram in Fig. 3 shows the change in oxygen tension in the brain of a rabbit whose abdomen had been exposed to 2500 r. After 2 hr there was virtually no reaction when oxygen was given, i.e. the level changed only insignificantly on breathing an oxygen-enriched mixture. Four hours after the exposure the "oxygen test" gave almost the same value as before irradiation. Only in one case in this series did we observe an increased oxygen tension exceeding normal 4 hr after abdominal radiation (rabbit No. 22, Table 2).

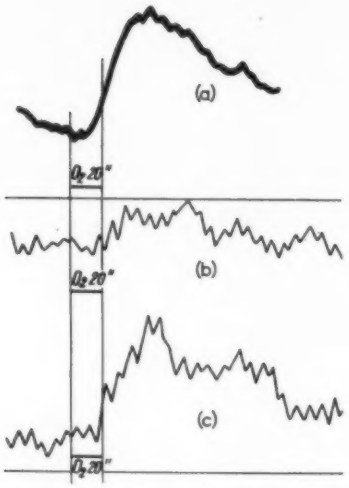

FIG. 3. Changes of the diffusion current during the "oxygen test" before and after exposure of the abdominal region. Rabbit No. 4. Signal = oxygen administration. (a) normal; (b) 2 hr after exposure; (c) 3 hr after.

TABLE 2. CHANGES IN OXYGEN TENSION IN THE BRAIN DURING THE "OXYGEN TEST" IN IRRADIATED ANIMALS EXPRESSED AS PERCENTAGE OF INITIAL VALUE

No. of rabbit	Radiation dose (r)	Area irradiated	After						
			5–10 min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
4	2500	Abdomen	78	—	25	80	90	—	84
17	2500	Abdomen	100	90	10	90	90	—	90
22	2500	Abdomen	64	84	68	—	180	—	140
23	2500	Abdomen	85	77	100	100	60	—	100
27	1100	Abdomen	56	87	78	57	56	56	43
30	1100	Abdomen	80	100	80	61	100	60	78

As a rule we first found an oxygen tension higher than normal a few days after the exposure. It then oscillated in a fashion similar to that in series 1 and 2.

In addition to changes in the absolute values of oxygen tension in the "oxygen test" we observed, as a result of X-irradiation, changes also of the background level of oxygen tension.

As previously described [16] the background level of oxygen tension in different rabbits is for an interval of a few minutes either absolutely constant, or it varies slightly about a constant level, showing waves of various frequencies and amplitudes. Total-body irradiation or head exposure seems to flatten out these periodic changes if they were present before the irradiation. The fluctuations began to vanish 5–10 min after the end of the exposure. About 2–3 hr after the exposure they had disappeared, but reappeared 6–7 hr after irradiation. In Fig. 4 we show the photograms of the change in the oxygen tension on the first day after exposure. This instability of the

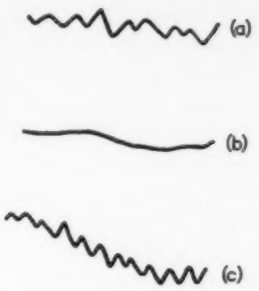

FIG. 4. Changes in the background oxygen level after whole-body exposure. (a) normal; (b) 7 min after exposure; (c) 6 hr after.

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background of oxygen tension remained for several days. Fig. 5 shows a characteristic change of the background of oxygen tension during the course of the radiation syndrome.

When a rabbit which normally showed fluctuations of the oxygen background is irradiated in the abdominal region, these fluctuations also normally disappear. It is, however, interesting that, in a series of experiments, as a result of the treatment, the fluctuations of background arose, or were sharply increased from an insignificant

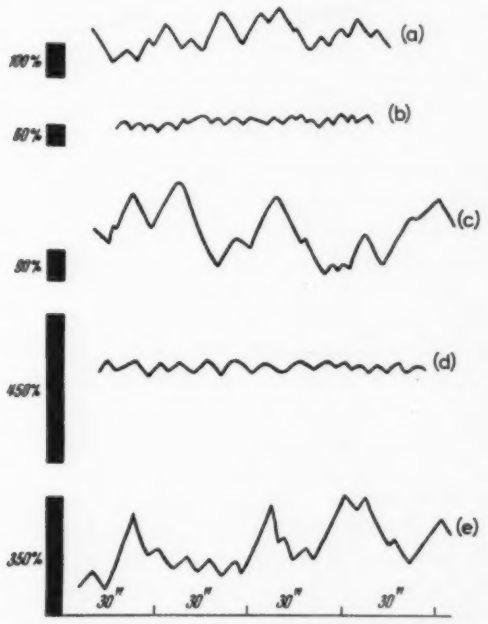

FIG. 5. Changes of the oxygen tension of the brain and of the background oxygen level at various intervals after X-ray exposure. Columns on the left: changes in oxygen-tension as percentage of normal. To the right of the columns: background oxygen level. (a) normal; (b) 2 hr after exposure; (c) 1 day after; (d) 33 days after; (e) 35 days after.

normal (Fig. 3). We have not yet established a relation between the absolute change of oxygen tension and the phenomenon of the changing fluctuations of the background. On the first and on later days the fluctuating character of the background disappears whether the oxygen tension is below or above normal.

Fig. 5 shows the change in oxygen tension (columns on the left) and the corresponding background level of oxygen tension in each case.

To elucidate the nature of the fluctuations of basal level we set up special investigations. We inserted a pair of electrodes into the brain to different depths. One was put 1 mm deep into the cortex, and the other 4–5 mm deep into the sub-cortical region. It was found that both the oxygen tension and the background fluctuations were different at different depths of one and the same zone of the brain. Fig. 6

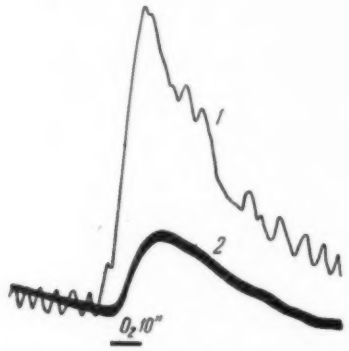

FIG. 6. Changes of the diffusion current during the "oxygen test" in the cortex and in the sub-cortical tissue. Signal = oxygen administration. Electrode (1) at 1 mm depth; electrode (2) at 4 mm depth.

represents the simultaneous photogram of two electrodes placed at different depths in the brain in an "oxygen test". Curve 1 shows the response of an electrode buried at 1 mm depth, and curve 2 shows that of an electrode at 4 mm depth. As a rule, the sub-cortical electrodes show background fluctuations more strongly. The ratio of the oxygen tensions in the cortex to those in the sub-cortical regions varies extraordinarily from animal to animal.

Another point of interest is the length of the period of enhanced oxygen content in the "oxygen test". In cases where the radiation disease develops acutely we observed some days before the death of the animal a change of configuration of the oxygen tension curve in the "oxygen test". On the seventh day after irradiation the utilization by the organism of the extra oxygen begins a minute or more after cessation of the administration of the oxygen, and the oxygen tension returns to its initial value only after 2·5 min (Fig. 7c) while in the normal, and a day after the irradiation, the time required for the utilization of the added oxygen was 1 min 20 sec (Fig. 7a, b). The rabbit was very sick that day; it had moist head and paws and breathed heavily, with crepitation. When the "oxygen test" was applied repeatedly, this recovery time was reduced. There arises a peculiar adaptation to oxygen loading, and Fig. 7(c) shows the response to the fourth of a series of oxygen tests made at 3 min intervals to be almost normal. Fig. 7(d) shows that on the eighth day the utilization time is

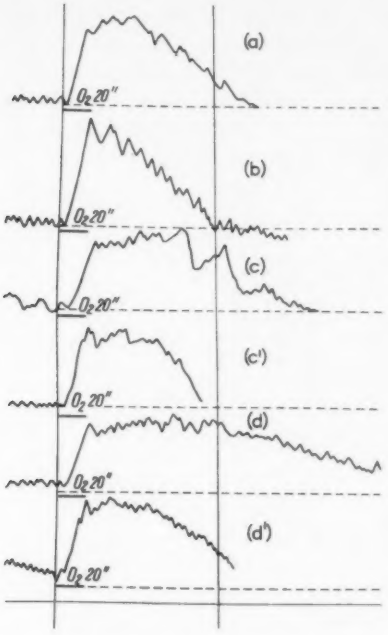

FIG. 7. Changes in the character of the curves during the "oxygen test" after irradiation. Signal = oxygen administration.

even more prolonged than on the seventh day. Nevertheless, repeated application of the "oxygen test" reduced the utilization time to normal even on the eighth day.

On the ninth day the rabbit was immobile and very close to death. It can be seen in Fig. 8 that the background as well as the response to the "oxygen test" had changed markedly. The background shows fluctuations of considerable amplitude with a slope to the right, and even repeated applications of the "oxygen test" failed to bring the oxygen tension curve, or the background, back to normal. The animal died that day.

As we pointed out in the introduction, our problem is to compare the observed changes of oxygen content of the brain with changes in blood circulation and respiration.

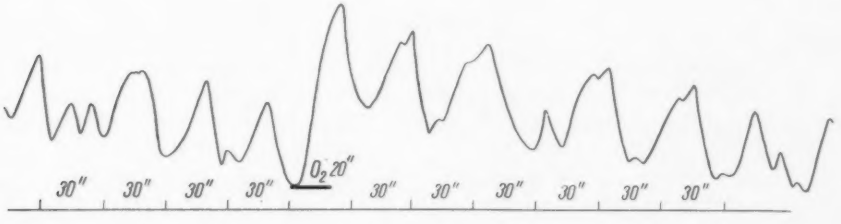

FIG. 8. Changes of the background oxygen level during the "oxygen test" at the final stage.

In Tables 3 and 4 we present our simultaneous observations on blood pressure, respiration rate, and oxygen tension.

As can be seen in Table 3 the blood pressure changed only 2 hr after irradiation and in all cases it was reduced. In the course of the first day the blood pressure fell continuously and remained low for the first two days after the exposure. When the abdominal of the stomach had been irradiated, blood pressure, in some cases, returned to normal values after 7 hr.

TABLE 3. CHANGES IN BLOOD PRESSURE AFTER X-IRRADIATION

No. of rabbit	Radiation dose (r)	Area irradiated	Initial blood pressure (mm Hg)	Blood pressure after exposure, after						
				1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
7	900	Whole body	106	106	100	90	70	70	—	65
5	1100	Whole body	110	105	95	80	70	65	65	—
24	1100	Whole body	105	105	103	90	—	65	—	60
13	1500	Whole body	112	110	105	90	90	85	80	—
10	1500	Head	115	100	60	60	60	—	50	—
12	3000	Head	110	90	85	80	60	50	—	40
27	1100	Abdomen	120	115	105	—	105	—	—	100
22	2500	Abdomen	112	110	—	90	—	85	80	—
4	2500	Abdomen	100	—	60	—	60	—	—	100

TABLE 4. CHANGES IN THE RESPIRATION RATE AFTER X-IRRADIATION

No. of rabbit	Radiation dose (r)	Area irradiated	Normal No. of breaths per min	Number of breaths per min after exposure, after						
				1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr
24	1100	Whole body	60	> 60	100	140	—	180	180	140
29	1100	Head	48	44	60	88	88	—	80	80
30	1100	Abdomen	40	50	—	48	44	—	40	44
22	2500	Abdomen	80	80	72	64	60	62	60	60
23	2500	Abdomen	120	130	—	160	160	—	120	110
27	1100	Abdomen	60	—	60	56	56	130	108	60

The respiration rate changed in no systematic way. The changes did not depend on the region of the body which had been irradiated.

DISCUSSION

The first point which draws our attention when we look at the results is the distinct difference in the change of oxygen tension during the first day in the brain of the rabbits that had either whole-body or head exposures and those of the rabbits that had an abdominal exposure only.

In the first two series, i.e. whole-body or head exposure, the change of oxygen tension in the brain, although showing individual variations in absolute magnitude and the time of appearance of the different phases, goes in the same direction. There was an increased oxygen tension for 2 hr after irradiation and sometimes for even

longer. These changes were appreciably larger in rabbits which had head exposures only.

It is quite probable that the initial temporary decrease in oxygen tension in the brain which we observed in some animals of the first two series always occurs. We would not have observed it if this initial depression happened during the exposure.

Animals which had only their abdominal region exposed showed an opposite change—a decrease in the oxygen tension in the brain. From the first minute after irradiation it remained low for the first 2–3 hr, sometimes longer, and then returned to normal.

The concentration of free oxygen in the tissue close to the cathode depends, in a whole living animal, on two factors—the blood supply, i.e. the delivery of the oxygen, and the rate of oxygen utilization by the tissue.

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Measurements of blood pressure and respiration made simultaneously with the "oxygen tests" showed that, in the first hours after irradiation, changes in blood pressure and respiration rate are in the direction opposite to the changes in the oxygen tension of the brain. In the first hours after irradiation, when the oxygen tension is high, there is a small fall of blood pressure which continues low for the first days. It is evident that the low blood pressure may help to a certain extent to bring the initially high oxygen tension in the brain back to normal values. But it seems unlikely that haemodynamic changes are alone in influencing the oxygen tension in the brain, because the rise of oxygen tension in the brain lasts 5–6 hr after irradiation, and sometimes shows even a second maximum at a time of constant low value, or even steady fall, of blood pressure. It is quite possible that the return to normal of the oxygen tension in the brain after the maximum is the result of the considerable fall of blood pressure, and does not represent a true return to normal oxidative processes in the tissue. It may be that the rate of oxidative reactions at this time actually remains at the same low level as before the blood pressure began to fall. No parallelism between the course of changes in oxygen tension and blood pressure are seen during the subsequent days.

Also rabbits whose abdominal regions only had been irradiated did not show any logical connection between the change in oxygen tension and observed changes in blood pressure. In this series of experiments we observed an immediate drop in oxygen tension in the brain, that either continued for the whole of the first day after treatment or reached a minimum at 2–3 hr with a subsequent return to the normal value in the course of the first day.

It can be seen in Tables 2 and 3 that the oxygen tension in rabbit No. 22 exceeded the normal value 4 hr after irradiation, while the blood pressure had fallen and continued to fall in the following hours. The oxygen tension in rabbit No. 27 dropped to less than half the normal value at about 6 hr, whereas its blood pressure dropped only very little; the blood pressure was 100 mm Hg at about 7 hr. A comparison of the changes of blood pressure with those of oxygen tension in rabbits whose abdominal regions have been irradiated leads to the conclusion that changes in oxygen tension are not determined by the changes in blood pressure.

From other workers' results [18] it is known that the mass of circulating blood, which determines the oxygen supply to tissues, is reduced in animals that have received either local or whole-body exposure to X-rays. These results also indicate

that the changes in oxygen tensions at certain phases are not parallel to the haemodynamic changes. However, it must be borne in mind that the volume of the circulating blood and the blood pressure do not entirely determine the blood supply of an organ. The final decision on this question must await more exhaustive information on the blood circulation in the brain itself.

As can be seen in Table 4, the changes in frequency of breathing did not in any instance proceed in the same sense as the oxygen tension. The considerable and rapid changes in the oxygen tension cannot be ascribed to changes in the frequency of breathing, since these did not coincide in time and direction with those of the oxygen. It should be added that according to Entina and Iakovleva [15] the frequency of breathing is not reflected in the oxygen tension.

From these considerations we may conclude that, taking into account the haemodynamics, the changes in oxygen tension in the brain of the irradiated animals of series 1 and 2 can be ascribed to a reduced oxygen utilization of the tissues. Immediately after the end of the exposure, conditions arise whereby the brain, which, despite the developing haemodynamic disturbances leading to a deterioration of oxygen supply, possesses an excess of oxygen—an indication that the processes of oxidation have been depressed.

One can imagine that in the case of whole-body or head exposure, local processes develop in the brain as a direct result of the X-ray exposure, and these may quite possibly be specific for brain tissue. The different reaction after exposure of the abdominal region only demonstrates the whole complex relationship between local processes resulting from direct irradiation, and reflex phenomena released by signals from the periphery.

The fact that when the abdomen alone is irradiated there is for some hours a super-normal oxidative activity in the brain tissue shows that we are dealing here with the reflex response to the irradiation of a peripheral region (manifested by increased oxygen usage) of a tissue protected from the direct action.

In the subsequent days, when secondary effects possibly connected with intoxication of the organism appear, all three series show the same considerable increase in oxygen tension.

The observation of reduced tissue respiration in rabbits is not easy to correlate with previous findings on whole organisms [19–21].

Other workers measured the gas metabolism of irradiated animals, mainly rats, and found that oxygen utilization increased after irradiation (Kitschner [19], Il'ina [20], Bystrov,* Barbashova [21]). This difference can be explained either by different experimental conditions, or by the fact that the determination of total gaseous exchange gives the total response and not values referring to individual tissues. But as Van Bekkum [4, 5], Mytareva [7], Voskoboinikov [8], have shown, respiratory inhibition after X-irradiation occurs only in certain organs.

In the rabbit with acutely developing radiation sickness after 3000 r to the head the response to the "oxygen test" became slower for some days before death. There was no rapid fall of oxygen tension on cutting off the oxygen supply. We cannot at the moment say why the response can be brought back to normal for a limited time by administering a small dose of oxygen with the air breathed by the animal. We do

* Read before the Radiobiological Conference, Leningrad, 1954.

not know whether, in this case, oxygen exerts its stimulating action via some or other brain centres, which then influence tissue respiration, or whether this is a direct effect of oxygen on the respiratory enzyme mechanism. Much is not yet clear as to the interrelation between the cortex, or some sub-cortical centres, on oxidative processes in the tissues, or as to the extent to which oxidation processes determine the condition of these centres. One fact is evident: just as a few minutes or hours after irradiation tissue respiration is disturbed, so in the development of the radiation syndrome many days after irradiation brain tissue respiration is considerably reduced.

The nature of the rhythmic changes in the basal level of oxygen tension is also not yet understood. But the fact that the fluctuations disappeared not only when the respiratory activity of the tissue was reduced, i.e. when we observed an increased oxygen tension (Fig. 5 on day 33), but also when the rate of oxygen utilization was above normal (Fig. 5 after 2 hr), together with our preliminary experiments on simultaneous determination of oxygen tension in cortex and sub-cortex, allows us to conclude that there must be various controlling factors operating on the oxygen level in the tissue. The oxygen tension and the rhythmic fluctuations differ in their relations to the radiation. Our preliminary measurements of the oxygen tension in the cortex and the sub-cortical region do not permit any definite conclusion as to the actual differences in the respiratory activity of different parts of the brain, as the values show a rather large variability. But they clearly show that considerable differences in the respiratory activity of different regions of the brain exist. This is confirmed by the recent findings of Heller and Elliot [22] and of Pope [23], who have shown that tissue respiration of brain tissue slices is different for different parts of the brain.

It will be necessary to establish an exact architectonic picture of oxidative processes in the brain and other organs and the degree of damage caused to them by variously localized irradiation. It will then be possible to distinguish between local, primary radiation damage and general regulatory damage, and to assess their relative importance in the development of radiation sickness.

CONCLUSIONS

1. X-irradiation of different parts of the body causes different changes of oxygen tension in the brain of the rabbit.

2. Total-body or head exposure results in an increased oxygen tension in the brain of the rabbit lasting for several hours after the end of the irradiation. This, taking into account the state of the blood pressure and the mass of circulating blood, indicates that radiation directly depresses respiratory activity in the cortical tissue of the brain.

3. Irradiating the abdomen of the rabbit results in a reduced oxygen tension in the brain, lasting for a few hours after the end of the exposure. This indicates a reflexly produced increase of oxygen consumption in the brain.

4. The changes in the oxygen tension produced in the brain by all forms of irradiation show a phasic character.

5. There are characteristic changes in the rhythmic fluctuations of the oxygen background level.

6. In the final stages of radiation sickness the response of the oxygen tension in the brain to added oxygen in the inhaled air is prolonged.

Translated by M. EBERT

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ON THE INFLUENCE OF IONIZING RADIATION ON THE METABOLISM OF PHOSPHORUS-CONTAINING COMPOUNDS IN THE ROOTS OF WHEAT

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(*Received* 14 *June* 1956)

THE influence of ionizing radiation on the metabolism of nucleic acids, phosphatides, phosphoric esters of carbohydrates, and other phosphorus-containing compounds has been studied carefully in animals [1–11].

In plants investigations of the disturbances of phosphorus metabolism are far from complete [12–19], the main attention having been directed to the metabolism of nucleic acids, and unrelated to the metabolism of other phosphorus-containing compounds. As a rule, large doses of radiation were used. Taking into account the exceptional importance of phosphorus-containing materials in the metabolism of plants, it seemed to us of interest to study the effects of relatively low doses of ionizing radiations on changes on the metabolism of nucleic acids, carbohydrate

phosphoric esters, phosphatides, and acylphosphates in plants. We believed that the elucidation of the initial interrelated changes in the metabolism of these compounds might help to throw some light on the mechanism by which ionizing radiations act in general.

Wheat of the variety Leutescens 062 was used. The grains were soaked and the roots of the seedlings were used four days later. This material is fairly uniform, easy to reproduce in a uniform physiological state, and it is sufficiently radio-sensitive. The growth conditions of the seedlings were standardized: 100–200 seeds were spread on canvas which was stretched over glass frames and kept in distilled water in a thermostat at 26° and in the dark. For irradiation the canvas supports with the seedlings were inverted so that the roots were exposed directly to the radiation. To prevent the roots from drying during irradiation they were covered with wet gauze.

Preliminary experiments were carried out with radiation doses of 1000 r (190 kV, 5 mA, cardboard filter, tube-to-object distance 30 cm). This dose sharply inhibited mitosis in the meristem of the young roots, but did not lead to rapid death.

We judged the intensity of the metabolism of the phosphorus-containing compounds by the rate of inclusion into them of radioactive ^{32}P from $Na_2H^{32}PO_4$.

The irradiated and the non-irradiated wheat roots were inserted for 1, 2, or 4 hr in $Na_2H^{32}PO_4$. The volume was 100 ml, the specific activity was 0·1–0·3 μ/ml and the pH 6·2. After the prescribed time the seedlings were washed in running water for 10 min, and then fixed in boiling acetone for 15 min. The roots were then cut off, dried at 60°, ground to a powder and dried to constant weight. Two samples of approximately 150 mg were taken from the experiment and the control for each analysis.

The 150 mg samples were refluxed for 5 min in a mixture of 15 ml ethyl alcohol and 5 ml ether in a 100 ml conical flask and filtered through a No. 4 sintered glass filter. The precipitate was returned to the same flask and refluxed for 30 min in a mixture of 10 ml methyl alcohol and 10 ml chloroform; it was again filtered off on the sintered glass filter, washed with ether, and the filtrates were combined and their volume made up to 50 ml. Two to three samples were taken for counting.

The dry residue was weighed and transferred to a centrifuge tube: 15 ml of cooled 7 per cent trichloroacetic acid were added in the cold and the mixture was allowed to stand, with stirring, for 30 min in the cold. This extraction with subsequent centrifuging was repeated twice more. The suspension was finally centrifuged, washed three to five times with 1 per cent trichloroacetic acid and three or four times with 10 ml cold water until neutral to litmus. The combined trichloroacetic extracts and the washings were made up to 100 ml and their radioactivity was measured. After that 20 ml of this trichloroacetic extract was neutralized with a 33 per cent solution of NaOH (eight to nine drops) using a universal indicator; 2·5 ml of magnesia mixture and 1·5 ml of 25 per cent ammonia solution were added to precipitate inorganic phosphorus and phytin. The solutions were left overnight and filtered through filter paper the next morning. The filtrate was diluted to 25 ml and its radioactivity was measured.

The dry residue from the trichloroacetic acid was weighed and incubated for 15–16 hr at 37° in 10 ml of 1N KOH. The suspension was centrifuged and the activity of the hydrolysate was measured. 5 ml of the hydrolystate were treated at

ice temperature with 1 ml 6N HCl and 5 ml cooled 5 per cent trichloroacetic acid. The turbid precipitate of DNA settled on standing in the cold for some hours; it was centrifuged and filtered through a No. 4 glass filter. The activity of the filtrate was then measured.

As the work involved both precipitates and solutions the activity of all fractions was expressed as counts per minute per mg dry weight of root. Since every experiment took 10 days, corrections for the decay of the ^{32}P had to be made. We carried out three experiments, each with two parallel variants, so that each estimated value was based, on an average, on 400 plants.

The results summarized in Table 1 show that 1 hr after irradiation the ^{32}P uptake is depressed in all fractions (nucleic acids, carbohydrate phosphoric esters, and in the mixed lipoid-carbohydrate fraction). Two, and especially four, hours after irradiation the picture changes considerably. ^{32}P uptake increased in the mixed lipoid-carbohydrate and in the carbohydrate phosphoric ester fractions by 30–40 per cent, while ^{32}P uptake in the nucleic acids) decreased to 50 per cent.

The drop in ^{32}P uptake 1 hr after the irradiation may be related to the decreased permeability of the irradiated roots to $Na_2H^{32}PO_4$, as we have shown previously [20]. The different ^{32}P uptake by different fractions 4 hr after irradiation, when permeability had been restored, might be explained by interference by the irradiation with the regulation of metabolic processes in the root tissues. We see that 4 hr after irradiation ^{32}P uptake into RNA and DNA is decreased, while that into phosphoric esters of carbohydrates and the mixed lipoid-carbohydrate fraction is markedly increased.

For a more detailed analysis of the mixed lipoid-carbohydrate fraction we applied the following procedure: 300–400 mg of dry ground roots of 400 plants, obtained after placing irradiated (1000 r) and non-irradiated seedlings for 1 or 4 hr in $Na_2H^{32}PO_4$ solution, were extracted for 6 hr with 100 ml ether in a Soxhlet apparatus. This extracted the free phosphatides. The extract was diluted to 100 ml and its activity was estimated by the counting of aliquot portions. The residue in the paper thimble was dried in air for 12 hr and refluxed in a 100 ml conical flask with 40 ml 80 per cent ethyl alcohol for 3 hr and filtered. This procedure hydrolysed lipoproteins and extracted carbohydrates of low molecular weight. The alcohol was filtered off through paper and the extraction was repeated with 30 ml 80 per cent alcohol for 1 hr, for the more complete extraction of the carbohydrates. The residue was filtered off on the same paper and washed free from adsorbed activity with hot ethyl alcohol. The combined extracts and washings were diluted to 100 ml and the activity determined in samples. It contained the phosphoric esters of the low-molecular weight carbohydrates.

The residue, with the filter, was refluxed in the same conical flask with 40 ml ether for 5–6 hr. This extracted the bound phosphatides. The suspension was filtered through paper, and the precipitate washed with 50 ml ether until the washings were free of activity. The ether extract was evaporated in a measuring flask to 10 ml and samples were taken for the activity measurement. Three such experiments, each with two parallel variants, were carried out; their results are shown in Table 2.

It can be seen that already 1 hr after irradiation ^{32}P uptake was markedly reduced in the free phosphatides, very strongly reduced in the bound phosphatides, but

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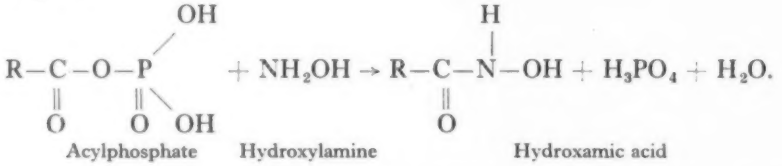
TABLE 1. THE EFFECT OF 1000 r ON THE ^{32}P UPTAKE INTO DIFFERENT FRACTIONS OF WHEAT ROOTS AT DIFFERENT TIMES AFTER EXPOSURE

Fraction	No. of expt.	1 hr after irradiation			2 hr after irradiation			4 hr after irradiation		
		Activity of dry roots imp/min/mg		Exptl. as percentage of controls	Activity of dry roots imp/min/mg		Exptl. as percentage of controls	Activity of dry roots imp/min/mg		Exptl. as percentage of controls
		Controls	Exptl.		Controls	Exptl.		Controls	Exptl.	
Lipoid-carbohydrate	60	182 ± 1	120 ± 8	65 ± 5·5	264 ± 20	218 ± 12	82 ± 6	—	—	—
	59	280 ± 10	180 ± 10	64 ± 6·5	252 ± 10	188 ± 8	71 ± 10	—	—	—
	61	153 ± 3	107 ± 2	69 ± 4	—	—	—	354 ± 3	450 ± 15	127 ± 5
Phosphoric esters of carbohydrates	60	1060 ± 50	816 ± 50	76 ± 9	1443 ± 30	1318 ± 50	91 ± 6	—	—	—
	59	777 ± 15	750 ± 25	96 ± 6	1144 ± 30	998 ± 70	87 ± 9	—	—	—
	61	480 ± 4	240 ± 3	50 ± 1	—	—	—	720 ± 8	980 ± 3	136 ± 2
RNA + DNA	60	1110 ± 10	925 ± 35	83 ± 4	2200 ± 40	1591 ± 20	72 ± 2·5	—	—	—
	59	3314 ± 30	2595 ± 40	75 ± 5	4272 ± 60	2990 ± 60	70 ± 2·5	—	—	—
	61	640 ± 10	544 ± 8	85 ± 2·7	—	—	—	2020 ± 12	1002 ± 10	49 ± 4
RNA	60	827 ± 20	724 ± 40	88 ± 7·5	1718 ± 60	1191 ± 15	68 ± 5	—	—	—
	59	3049 ± 40	2390 ± 35	78 ± 3	3556 ± 40	2550 ± 50	72 ± 2	—	—	—
	61	500 ± 4	440 ± 4	88 ± 2	—	—	—	1320 ± 10	674 ± 6	52 ± 1
DNA	60	283 ± 10	201 ± 5	71 ± 4·6	618 ± 20	400 ± 5	64 ± 3	—	—	—
	59	265 ± 10	202 ± 5·3	76 ± 5·3	716 ± 20	460 ± 10	64 ± 3·5	—	—	—
	61	140 ± 4	104 ± 4	74 ± 4	—	—	—	700 ± 2	328 ± 4	47 ± 0·5

hardly changed in low-molecular carbohydrate phosphoric esters. Four hours after irradiation, ^{32}P uptake was increased by 20 per cent in the phosphoric esters of the carbohydrates and in the free phosphatide fractions, whereas it was reduced by 60 per cent in the fixed phosphatide fraction. This is in agreement with the data in Table 1.

Comparing the results of Tables 1 and 2 it can be said that the synthesis of high-molecular compounds, such as complex proteins, nucleoproteins and lipoproteins, in the roots of the plants is depressed by irradiation at 1000 r. In contrast to this the synthesis of low-molecular compounds, namely carbohydrate phosphoric esters and free phosphatides, is not depressed; the activation of their synthesis a few hours after irradiation is apparently bound up with the formation of an excess of phosphate as a result of the slowing of the synthesis of the high-molecular compounds.

Energy-rich phosphate compounds, such as acylphosphates, play an important part in carbohydrate metabolism. We have observed a stimulation of the metabolism of low-molecular carbohydrates 2 to 4 hr after irradiation. For this reason it was interesting to follow the concentration of acylphosphates in the roots at the same times after irradiation. For this we adopted the method of Lipmann [21] which is based on the formation of hydroxamic acid in the action of hydroxylamine on acylphosphates.



The amount of hydroxamic acid was estimated colorimetrically. It forms a cherry-red complex with ferric ions. It must be pointed out that the sensitivity of this method is considerably less than that of the isotopic methods with ^{32}P.

For the estimation of acylphosphates we used 2 g of wet roots. These were dried on filter paper and quickly ground up in a previously prepared mixture of 3 ml of acetate buffer, pH 5·4, 0·5 ml of a solution of 28 per cent hydroxylamine hydrochloride, 0·5 ml of a 14 per cent NaOH solution, and quartz sand.

The reaction takes time; so 15 min from the beginning of the grinding must be allowed to pass. Then 4 ml of 12 per cent trichloroacetic acid were added and the mixture centrifuged for 10 min. Two ml 5 per cent $FeCl_3$ in 1N HCl were added to 4 ml of the filtrate, and the red colour measured on the photoelectric colorimeter using blue filter No. 7. As a control, we used the blank reaction of the reagents and also the reaction of the root tissue without the addition of hydroxylamine since the tissue extract itself was lightly coloured. In Table 3 we give results (expressed as extinction values E_0) on the effect of irradiation of various doses—on the increase with time of substances of the acylphosphate type.

It can be seen that immediately after irradiation with 1000 r and 3000 r the acylphosphate contents of the roots had not changed, but 2 hr after 1000 r it had increased by 40 per cent and 4 hr after 3000 r it had risen by 70 per cent. The amount of acylphosphate was back to normal values 24 hr after 1000 r. These results are in agreement with the activation of the metabolism of carbohydrate phosphoric

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TABLE 2. THE EFFECT OF 1000 r ON THE ^{32}P UPTAKE INTO DIFFERENT FRACTIONS OF WHEAT ROOTS AT DIFFERENT TIMES AFTER EXPOSURE

Fraction	No. of expt.	1 hr after irradiation			4 hr after irradiation		
		Activity of dry roots imp/min/mg		Exptl. as percentage of controls	Activity of dry roots imp/min/mg		Exptl. as percentage of controls
		Controls	Exptl.		Controls	Exptl.	
Ether I (free phosphatides)	62	108 ± 3	67 ± 3	62 ± 5	—	—	—
	63	102 ± 3	55 ± 1	53 ± 5	245 ± 10	343 ± 12	147 ± 3·5
	64	115 ± 0	84 ± 5	73 ± 4·5	204 ± 5	230 ± 5	112 ± 4
	Mean	—	—	62 ± 3	—	—	129 ± 3·5
Alcohol (phosphoric esters of carbohydrates)	62	374 ± 10	352 ± 2	94 ± 3·5	—	—	121 ± 10
	63	494 ± 20	534 ± 5	108 ± 5	1070 ± 40	1298 ± 60	129 ± 6
	64	418 ± 6	314 ± 7	75 ± 3·5	1185 ± 20	1530 ± 50	125 ± 8
	Mean	—	—	92 ± 3	—	—	—
Ether II (bound phosphatides)	62	37 ± 1	15 ± 1	40 ± 1	—	—	—
	63	57 ± 1	33 ± 1	58 ± 2	53 ± 3	27 ± 2	50 ± 8
	64	—	—	49 ± 1	23 ± 0	8 ± 0	34 ± 0
	Mean	—	—	—	—	—	42 ± 2

TABLE 3. THE EFFECT OF DIFFERENT DOSES OF RADIATION ON THE ACYLPHOSPHATE LEVELS AT DIFFERENT TIMES AFTER EXPOSURE (E_0—COLORIMETER EXTINCTION VALUES)

Time after irradiation (hr)	E_0 of unirradiated controls	1000 r			3000 r		
		E_0	Increase as percentage of controls	Mean increase (per cent)	E_0	Increase as percentage of controls	Mean increase (per cent)
0	0·78 ± 0·02 (mean of 12 observations)	0·74 ± 0·02	—	—	0·76 ± 0·02	—	—
		0·72 ± 0·03	—	100	0·76 ± 0·01	—	100
		0·78 ± 0·02	—	—	—	—	—
		1·10 ± 0·03	140	—	—	—	—
		1·15 ± 0·01	147	146	—	—	—
2		1·23 ± 0·02	158	—	—	—	—
		1·09 ± 0·04	140	—	—	—	—
4		—	—	—	0·99 ± 0·02	127	127
6		—	—	—	1·35 ± 0·01	173	181
		—	—	—	1·48 ± 0·02	190	—
		0·78 ± 0·02	—	—	—	—	—
24		0·73 ± 0·04	—	100	—	—	—

esters observed 2 and 4 hr after irradiation (Table 2). It should be noted, however, that the colour reaction used can also be produced by peroxides, as Kuzin and Shkolnik [22] have shown.

CONCLUSIONS

1. One hour after wheat roots were exposed to 1000 r X-radiation ^{32}P uptake into nucleic acids, into phosphoric esters of carbohydrates, into free and bound lipoids, was reduced. This reduction can be explained by a lack of $Na_2H^{32}PO_4$ in the irradiated roots, as the total absorption of ^{32}P by the roots 1 hr after irradiation was reduced.

2. Two and four hours after 1000 r the roots absorbed ^{32}P at the normal rate. ^{32}P incorporation into nucleic acids and into bound phosphatides remained considerably lower than normal, whereas incorporation into the esters of carbohydrates, and into free phosphatides, increased considerably.

3. Four hours after 1000 r, the regulation of synthetic and breakdown processes was disturbed. High-molecular compounds, such as nucleoproteins and lipoproteins, showed a decreased rate of synthesis, whereas low-molecular compounds, such as phosphoric esters of carbohydrates and free phosphatides, showed an increased rate of synthesis.

4. Together with the metabolism of nucleoprotein, that of lipoproteins is fairly radio-sensitive since ^{32}P uptake into bound phosphatides is decreased 2–4 hr after 1000 r by 50 per cent.

5. Two hours after 1000 r, and 4 hr after 3000 r, acylphosphate levels increased considerably in the roots. This agrees well with the increased rate of production of phosphoric esters of carbohydrates at the same time interval after irradiation.

Translated by M. EBERT

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ON AN ANOMALOUS REACTION CAUSED BY SMALL DOSES OF RADIATION

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(*Received* 16 *April* 1956)

1. INTRODUCTION

It is generally known that the relationship between a dose of irradiation and its effect on a biological reaction may be described either by an exponential curve or by a curve resembling that of the Poisson or Gauss distribution functions [1, 2]. The same is true of reactions caused by other damaging agents such as poisons [3, 4]. The theoretical explanation of the nature of this relationship has been the subject of discussions over many years, and we shall not raise this question here, beyond referring to the above-mentioned monographs. In the present communication we shall deal with an interesting case of deviation from the usual relationship, and shall try to advance a biophysical explanation for it.

Chromosome disruption is one of the best-studied radiobiological reactions. Many experiments [5] have shown that the number of chromosome disruptions or translocations is directly proportional either to the irradiation dose or to the square of the dose. Similar results had been obtained by us in experimental irradiations of pea seeds and seedlings by either X-rays or gamma-rays. However, in experiments where the seeds were soaked in an unfractionated solution of uranium fission products, a different relationship between dose and effect was observed. This relationship followed a straight line, intersecting, on extrapolation, the ordinate axis above the origin. Similar curves have been obtained by other workers, but usually the observed effect has not been considered significant, and has been regarded either as spurious [6] or as arising from an insufficiently exact control reaction [7]. Bonnier [8–10] was the first to give serious attention to this effect, to prove its reality, and to attempt an explanation. His tentative suggestion was that this effect is due to the formation in the protoplasm, on irradiation, of chemical substances producing the same effect as direct ionization. Below we are presenting the results of our own experiments and a discussion of the possible causes of the phenomenon.

2. EXPERIMENTAL

(*a*) *Methods.* The experiments were carried out on peas of the "Capital" variety from the Cheliabinsk Seed-selection Station.' The source of radiation was an old, unfractionated solution of uranium fission products, consisting chiefly of ^{144}Ce, ^{90}Y, ^{144}Pr, ^{147}Pm, ^{90}Sr, ^{137}Cs, ^{106}Ru, and ^{106}Rh; the content of other radioisotopes was less than 1 per cent. Such a solution acts as a practically pure source of beta-radiation. According to a calculation performed by N. V. Gorbatiuk, 25 g of seeds soaked, in a 120 ml round-bottomed flask, in a solution of specific activity of 1 mc

per litre receive 24·5 rep of beta-radiation and 0·4 rep of gamma-radiation in the course of 24 hr. In our experiments solutions of specific activity 0·25 to 640 mc per litre were used. The dry seeds were placed in a solution of suitable concentration for 24 hr, and were then transferred into Petrie dishes containing moist sand, and left to sprout. Seeds soaked in tap water or in a solution of stable isotopes served as controls (no difference between the two was observed). Two to four days after the end of the soaking period the root tips were fixed in Carnoy's mixture for cytological analysis. The investigation was carried out on temporary preparations stained with acetocarmine or acetolacmoid, and the percentage of anomalous anaphases and telophases was determined. Cells containing fragmented chromosomes or chromatin bridges were regarded as anomalous. Fifty cells were analysed in each preparation and the total number analysed in the experiments described here was 7616.

(*b*) *Results of experiments.* The results obtained are represented graphically in Figs. 1 and 2, plotted logarithmically, since on the logarithmic scale exponential curves straighten out. Our experiments were conducted within very wide limits of concentration—the highest being 2560 times greater than the lowest—so that the results for high concentrations and those for low ones have to be considered separately. The data for high concentrations—40 to 640 mc per litre—are given in Fig. 1. and, as may be seen, the points correspond well to an exponential relationship, lying along a straight line when plotted logarithmically. The straight line, plotted according to the least-squares method, is described by the equation

$$\ln (N_0 - N_c) - \ln N_0 = \ln 0{\cdot}847 - 0{\cdot}94\, c, \qquad (1)$$

where N_0 is the total number of cells investigated, and N_c is the number of cells found to be anomalous at concentration c in curies per litre.

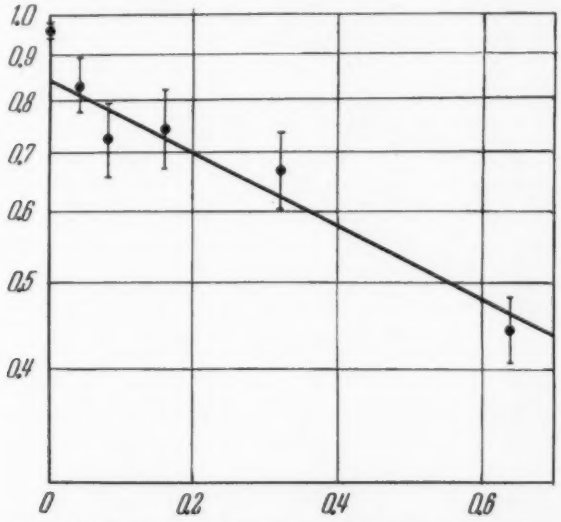

FIG. 1. Influence of high concentrations of fission-product mixture on mitosis in root meristem of peas. Along the abscissa—concentration of the solution in curies per litre; along the ordinate—ratio of the number of normal anaphases and telophases (having no bridges or fragments) to the total number. The points are plotted with triple error; the curve is drawn according to the method of least squares.

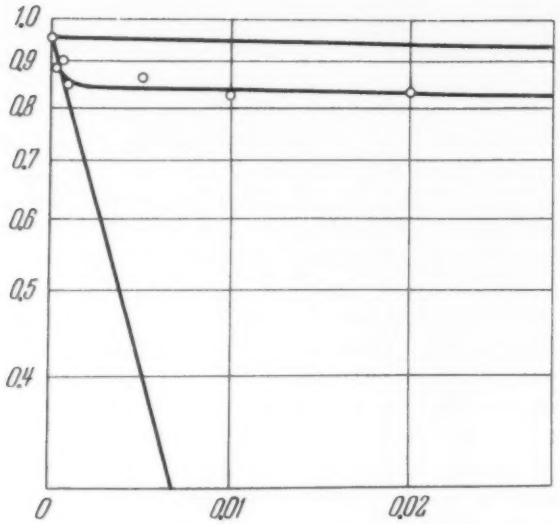

FIG. 2. Influence of low concentrations of fission-product mixture on mitosis. Legend, same as for Fig. 1. The straight lines are the exponential components, into which the curve is resolvable.

Converting the equation for the curve into normal co-ordinates:

$$N_c/N_0 = 1-0{\cdot}847e^{-0{\cdot}94c}. \tag{2}$$

According to this formula at zero concentration there should be 15·3 per cent anomalous cells, but in our controls there were observed only $4{\cdot}1 \pm 0{\cdot}63$ per cent such cells. This deviation is so great that it cannot be considered fortuitous. Indeed, experiments with lower concentrations show that the relationship is not, in fact, a simple exponential one. In Fig. 2 the results for concentrations of 0·25–20 mc per litre are given. Analysis of the results reveals that from a concentration of 1·0 mc per litre onwards the relationship between effect and concentration follows equation (1); only points corresponding to concentrations of 0·25 and 0·5 mc per litre do not exhibit this relationship and give a lower effect than that expected from equation (1). Graphic analysis shows that the general curve is composed of two exponential ones. The general equation for the dependence of the effect on the concentration may be represented as

$$N_c/N_0 = 1-0{\cdot}959\,(0{\cdot}88e^{-0{\cdot}94c}+0{\cdot}12e^{-167c}). \tag{3}$$

Thus, in our case we are dealing with an obviously complex relationship. We shall return to a possible explanation of our results after a theoretical discussion of processes capable of giving rise to such curves.

3. THEORETICAL

Composite curves may only be obtained in those cases where a given effect results from the participation of several processes. The following possibilities appear to us to be the most feasible:

(1) Different radiosensitivity of the individuals being irradiated.

(2) Identical radiosensitivity of the individuals irradiated, the curves under discussion arising, presumably, under one of the following circumstances:

A. The individuals being irradiated acquire different radiosensitivity as a result of the irradiation itself. It may be envisaged, for instance, that an organism non-reactive to a small dose becomes more sensitive to a subsequent irradiation. However, this effect would be more likely to come into play with large doses. It may be that this particular phenomenon does explain certain deviations from direct proportionality in the case of large doses. Since, however, we are interested in processes occurring at very low doses of irradiation this case will not be considered in detail further.

B. One and the same effect may occur as a result of different processes. This case deserves especial attention since it is known that the biological effect of ionizing radiations is connected not only with direct ionizations, but also with the formation of products of radiolysis of water and, possibly, with atomic excitation.

C. One and the same ultimate effect may occur with primary damage of different parts of the cell.

D. The same effect may result from secondary interactions. Thus two primary changes may occur, neither of which by itself could lead to the observed effect, but could do so conjointly. Such phenomena may be expected particularly in the elucidation of genetic effects. These phenomena, like those of group A, may only be observed with large doses of radiation and thus will not be specially considered.*

We chose the following simple method for the analysis of these possibilities. In the case of an exponential relation the logarithm of the effect bears a linear relationship to the dose. Thus, considering the "basic" component, significant at high dosages, as depending exponentially on the dose, it is easy to obtain the point of intersection of the curve, extrapolated from high doses, with the ordinate. Clearly, in the event of a different relationship between the "basic" component and the dose, by application of the corresponding transformations the analogous results can be obtained.

The following expression, based on the theorem of the summation of probabilities, is true for the first hypothesis in the simplest and the most likely case, viz. when for all the individuals concerned, the effect depends exponentially on the dose:

$$N_D/N_0 = n_1\,[1-\exp\,(-k_1D)]+n_2\,[1-\exp\,(-k_2D)], \tag{4}$$

where $n_1+n_2 = 1$ and $k_1<k_2$.

At high doses $\quad 1-N_D/N_0 \approx n_1 \exp\,(-k_1D)$

and $\quad \ln\,(1-N_D/N_0) \approx \ln n_1-k_1D.$

At zero dose $\ln\,(1-N_D/N_0) \approx \ln n_1$, but since $n_1<1$, a positive effect, on extrapolation, corresponds to the zero dose. Using any other function of the dose as the second term in the above addition leads to the same result.

An identical mathematical expression describes cases B and C of the second

* After the completion of the present work, a paper by Harm and Stein [11] appeared analysing the possibility of a saturation effect at high doses of radiation. The authors consider cases corresponding to hypotheses A and D, as well as the assumption that mutagenic substances may be destroyed by radiation, and conclude that in all these instances the saturation effect may occur. The whole argument is carried out with reference to the particular case of the production of mutations, i.e. the dependence on the dose of the ratio of the number of reacting entities to the number of surviving ones, and not to the number of irradiated ones. This publication to a certain extent amplifies our conclusions.

hypothesis. In the case of two exponential relationships the theorem of the multiplication of probabilities gives:

$$1-N_D/N_0 = \{1-[1-\exp(-k_1D)]\}\cdot\{1-[1-\exp(-k_2D)]\} = \exp[-(k_1+k_2)D].$$

Consequently, on this assumption an exponential curve is obtained differing in slope from the original one.

If the complementary component is of the S-form, then

$$1-N_D/N_0 = \{1-[1-\exp(-k_1D)]\}\cdot\{1-[-\exp(-k_2D)]^m\}, \quad (6)$$

where $m>1$. Expansion of the binomial and algebraic manipulation gives:

$$1-N_D/N_0 = m\cdot\exp[-(k_1+k_2)D]-0{\cdot}5\,(m^2-m)\exp-[(k_1+2k_2)D]+ \\ +\ldots\pm\exp[-(k_1+mk_2)]D. \quad (7)$$

At high dosages $1-N/_DN_0 \approx m\cdot\exp-(k_1+k_2)D$

and $\ln(1-N_D/N_0) \approx \ln m-(k_1+k_2)D.$

At zero dose the ordinate of the straight line is equal to $\ln m$. But since, by definition, $m>1$, any curve is an S-form one and the observed effect cannot occur.

Finally, a possible case may be when the complementary component has an asymptote less than unity. Then

$$N_D/N_0 = 1-[\exp(-k_1D)]\cdot\{1-a[1-\exp(-k_2D)]\}, \quad (8)$$

where $a<1$—the asymptote to which the complementary component is tending. On simplifying, equation (8) becomes

$$1-N_D/N_0 = (1-a)\exp(-k_1D)+\exp[-(k_1+k_2)D].$$

Analogously to the preceding, the following is true for high doses

$$\ln(1-N_D/N_0) \approx \ln(1-a)-k_1D.$$

Extrapolation confirms that on a ln/ln scale the straight line intersects the ordinate axis at point $\ln(1-a)$. Thus a curve of the type that interests us is obtained. The question of what biophysical meaning the equation obtained may have will now be considered. In case B it may mean that in the irradiated tissues a product is accumulated whose concentration becomes stationary at even small doses. But if the concentration is sufficient to cause a reaction in some of the cells, it is difficult to understand why all the cells do not react at this concentration. To explain this contradiction, it must be assumed that either not all the cells react at a stationary concentration, or that not all the cells are sensitive to the given product, i.e. to return to the first hypothesis. In case C it also leads to a recognition of the heterogeneity of the cells being irradiated. Thus, the conclusion must be made that on irradiation of homogeneous material curves showing an anomalous reaction at low dosages cannot indicate a different mechanism of reactions leading to one and the same effect. Naturally, this does not constitute a proof of the absence of such differences of mechanism. On the contrary, a whole series of facts indicate that together with the direct ionization of biologically important molecules and structures their secondary interactions with products of radiolysis of water play a significant part [12]. However, the above calculations show that this phenomenon cannot affect the shape of the curves of the dosage effect, in the sense of a disproportionate effect at low dosages.

Although the analysis performed for fundamental cases shows that of all the simple hypotheses only the one involving the heterogeneity of the irradiated material gives curves with an anomalous reaction at low doses, yet sometimes this explanation

does not seem to be entirely feasible. This is so particularly in reference to the experiments of Bonnier, mentioned previously, on the induction of mutation by X-rays. His results are more readily accounted for in another way by a particular case of hypothesis B. This case is especially interesting from a biophysical viewpoint, and will therefore be considered further below.

It is well known that many reactions induced by radiation also occur spontaneously. Timoféef-Ressovsky *et al.* [2] have shown in the case of mutations that such reactions can follow the Arrhenius-van't Hoff rule, the rate of the spontaneous reaction thus being determined by the Boltzmann distribution. Strictly speaking, all cases of induced reactions involve a summation of the original energy of the reacting molecule with the energy imparted. However, the original energy may be disregarded when the energy imparted exceeds considerably the energy of activation (which occurs almost always in ionizations). If the imparted energy is less than the energy of activation (which may occur on atomic excitation or in free radical reactions) then only those of the affected molecules will react whose initial energy is sufficiently high. Thus, if the rate of a spontaneous reaction is

$$v = z = z_0 \cdot \exp(-E/RT),$$

then the rate of the reaction induced by an agent imparting little energy will be

$$v = z_0 \cdot \exp[-(E_0-\varepsilon)/RT], \tag{9}$$

where z is the number of effective collisions, z_0 is the total number of collisions, E is the energy of activation, ε is the energy imparted, R is the gas constant, and T is the temperature.

In this way, for an agent possessing an energy lower than the energy of activation, any collection of objects being irradiated is nonhomogeneous. Hypothesis B can now be analysed taking into account this additional condition. If N_1, N_2, and N_3 denote the number of units reacting at a given dose at the expense of an agent of energy higher than the energy of activation, of an agent of lower energy, and of a spontaneous process, respectively, then in the simplest case

$$N_1/N_0 = 1-\exp(-k_1D),$$
$$N_2/N_0 = A\,[1-\exp(-k_2D)],$$
$$N_3/N_0 = C = \text{const.},$$

where A is the probability obtainable from (9). For the total number of reacting units

$$N_D/N_0 = 1-(1-N_1/N_0)\cdot(1-N_2/N_0)\,(1-N_3/N_0) =$$
$$= 1-\{1-[1-\exp(-k_1D)]\}\cdot\{1-[A-A\cdot\exp(-k_2D)]\}(1-C) =$$
$$= 1-(1-C)\,\{(1-A)\cdot\exp(-k_1D)+A\cdot\exp[-(k_1+k_2)\,D]\}. \tag{10}$$

Thus, an equation of the type of (4) is arrived at, i.e. an anomalous reaction should be observed at low dosages.

4. DISCUSSION

In discussing the experimental results it must be borne in mind that the curve obtained for the dependence of the number of anomalous mitoses on the dose is an exception to the general rule and therefore it is obvious that the result obtained must be explained by the experimental conditions. These differed from the usual ones, firstly, in the irradiation being done with beta-rays with an average energy of the

order of 1 MeV instead of the usually employed X-rays or gamma-rays; secondly, in the irradiation being effected by soaking in a radioactive solution and not from without; and lastly, in the irradiation being unusually prolonged. The first two circumstances appear to be non-significant both from general biophysical considerations, and also because even if it is assumed that the effect is caused precisely by these factors it is difficult to understand why these conditions should have affected so markedly only certain of the cells. As regards the duration of irradiation, its influence is quite explicable. If it is considered that the radiosensitivity of the cells undergoes cyclic changes (this has been proved in the case of mitotic cycles [13]), with the cells becoming much more sensitive over a definite short period of time, then naturally on prolonged irradiation a larger number of cells is irradiated in this sensitive phase. The correctness of this explanation is confirmed by some, so far not very numerous, data on the cytological effects of very prolonged external irradiation. Koller [14, 15] has found that, on uninterrupted irradiation of very low strength, the effect ceases to depend on the dose, and becomes a function of the duration of the irradiation. Thus, in *Tradescantia*, chromatid disruptions are caused by a dose of 50 r, given over 500 min, but the same or even twice as large a dose given over 200 min produces no such effect. Koller also notes an increase in the rate of cell division on prolonged irradiation, but not on short irradiation with the same doses. Evidently irradiation of cells which are in an unusual state may lead to qualitatively new effects. It is clear that on prolonged irradiation these effects will be observed more frequently, firstly because of the above-mentioned cyclic changes in sensitivity and, secondly, because the irradiation itself may alter the properties of the objects being irradiated.

We encountered a similar effect in experiments with prolonged external irradiation with gamma-rays. This phenomenon, which we call superfragmentation, is characterized by a disruption of chromatin in isolated cells into dozens of fragments. Superfragmentation is observed exceptionally in ordinary irradiations, and then depends on the dose. If germinating seeds are irradiated for 24 hr, this phenomenon becomes usual, and practically independent of the dose. With a dose of 5000 r superfragmentation is observed in 22 per cent of the cells, and with a dose of 250 r in 18 per cent. If the same doses are given over 6 hr or 30 min, then with a dose of 250 r this phenomenon occurs in approximately 1 per cent of the cells and only with 5000 r reaches approximately 10 per cent. Prolonged or fractionated irradiation is also characterized by definite qualitative peculiarities, which have been described by us [16].

The biophysical analysis carried out in the present communication shows that the anomalously marked reaction at low dosages of irradiation indicates the non-homogeneity of the irradiated material. In some cases, e.g. the experiments of Beam on the irradiation of resting and of budding yeast cells [17], this conclusion from an analysis of the curves is trivial. However, the nature of the non-homogeneity is not always as simple as that. Thus, in our experiments it must be interpreted as follows: at a particular stage, lasting a short period of time, the cells become 100–200 times more sensitive than usual, possibly under the influence of the irradiation itself. In the experiments of Bonnier, the unusual shape of the curves is most readily explained by the effect being caused by several agents of different energy. This supposition

can easily be verified experimentally since, if it is correct, the effect should, for example, be temperature-dependent.

It must be emphasized that the conclusions reached hold only for relatively simple reactions and for the action of low doses of radiation. Reactions indirectly due to a series of primary changes may be explained otherwise as well. This applies, in particular, to the influence of radiation on the growth of plants, which is made up of at least two different and occasionally opposite effects, e.g. the action on the rate of mitosis, and the fragmentation of chromosomes, often leading to the death of the cells [18]. The disproportionate effect at high dosages, not infrequently observed in experiments, especially in ultra-violet irradiations, may also be otherwise explained; for instance, according to the hypotheses A and D.

In conclusion it must be noted that in the field of low doses and of low intensities new and interesting phenomena may be expected. This follows from theoretical considerations. Recently Shekhtman [19] has shown that theoretically, in some cases, a "tolerance intensity" may be expected, below which no dose will produce any effect. This has been observed experimentally. Koller, in the above-mentioned experiments, has found that below a certain intensity of the dose irradiation does not suppress cell division. In the present work, on the contrary, is shown the theoretical possibility of a disproportionately high effect of small doses, on prolonged irradiation, and this possibility is confirmed experimentally. These results have practical as well as theoretical importance, since they demonstrate that unmodified extrapolations from high to low doses and intensities are not permissible. In this connection, the theoretical and experimental investigation of the biological action of small doses and prolonged irradiations is of great interest.

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CONCLUSIONS

(1) Pea seeds were soaked for 24 hr in solutions of uranium fission products. The dependence of the number of anomalous mitoses (bridges, fragments) on the concentration gives a supersaturated curve, resolvable into two components.

(2) Theoretical analysis shows that such curves may be obtained only with non-homogeneous material. The action of several agents producing the same effect on a homogeneous population gives simple exponential or S-shaped curves. Our experimental results may be explained on the assumption that the sensitivity of the cells undergoes cyclic changes. On prolonged irradiation a greater number of cells is irradiated in the hypersensitive state.

(3) The possibility of the action of low-energy agents (having a lower energy than the energy of activation) is analysed. In this case the energy of the agent is added to the energy of thermal oscillations, decreasing the index of power in the Arrhenius-van't Hoff equation. In the case of conjoint action of agents of a high energy (ionization) and of low energy (excitation or free radicals) composite curves must be obtained. The results of Bonnier may be thus explained, and an experimental check of the hypothesis is proposed.

Translated by I. GORE

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CALCULATION OF THE INTENSITY OF THE ALPHA-RADIATION DOSE ARISING FROM A RADIOACTIVE SUBSTANCE DISTRIBUTED INSIDE THE ORGANISM

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(*Received* 1 *March* 1956)

THE complexity of the calculation of the intensity of the dose arising from the introduction of radiating substances into the organism is mainly due to the non-uniform distribution of the substance in the tissue and to the heterogeneity of the tissue medium itself with respect to screening power.

The number of publications in this field is small. The work of Spiers is probably the most significant [1], although in his investigations the author does not go beyond calculations for the simplest case, viz. a flat interface between two media—bone and soft tissue. Since in reality interfaces between different tissues have finite, arbitrarily changing, curvature, the case of a flat interface is not always a sufficiently close approximation to the actual tissue interfaces.

In solving, under more general conditions, the problem considered by Spiers and by Munson [1–3], we are also dealing with the cases of a spherical or a cylindrical interface between two media. On this basis it may be possible to approach closer to the solution of this problem of strength of dose under actual conditions.

The authors of a number of publications consulted mainly pursue the aim of

solving this or that problem on the distribution of the intensity of alpha-radiation doses in tissues in relation to definite experimental conditions or clinical problems. These publications, valuable in themselves, and possessing some elements of general interest, cannot, on account of the limitations of the questions studied, provide a basic method for the solution of problems of intra-tissue alpha-dosimetry. The need for such a method is becoming more and more obvious, as the number of biological and medical problems requiring it increases.

We are far from claiming to be able to provide an exhaustive method of such calculations, but in the field of the questions considered below we have attempted to marshal the results so as to form a whole, and to be applicable to the solution of a wider circle of problems of intra-tissue alpha-dosimetry. The first section of the present communication (Section 1) deals with the calculation of the mean intensity of the dose in border areas of two media, divided by a flat, spherical or cylindrical interface; the second (Section 2) is concerned with the distribution of the intensity of the dose within the border areas of two media, separated by a flat or a spherical interface; and the third (Section 3) presents results of an experimental check of certain theoretical conclusions and a comparison of our results with those of Spiers.

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The dose in any medium is the energy absorbed from the transported ionizing radiation. Thus, the problem of the distribution of a dose must be solved by means of equations describing transport of energy and its absorption in a medium. These equations, by their very nature, must possess a large measure of universality.

Now a flow of energy being carried by alpha-particles from medium i into medium k across an interface σ, may be determined by integrating the following equation:

$$E_i = \iint_{\sigma} d\sigma \iiint_{V_i} \frac{\cos \vartheta}{4\pi r^2} N_i E_\alpha \left(1 - \frac{r}{R_i}\right) r^2 \sin \vartheta \, dr \, d\vartheta \, d\varphi, \tag{1}$$

where N_i is the number of alpha-particles arising in a unit of time in a unit of volume of medium i; E_α is the total energy of an alpha-particle; R_i is the length of the path of an alpha-particle in medium i; V_i is the volume of medium i in which the alpha-particles arise that pass into medium k across the differential element $d\sigma$ of the interface, σ; $\left(1 - \frac{r}{R_i}\right)$ is the factor determining the amount of energy transported by an alpha-particle from medium i to medium k across the element $d\sigma$; cos θ describes the dependence of the transfer of energy on the angle of incidence of the differential flow on the element $d\sigma$; $\frac{1}{4\pi r^2}$ is a factor, describing the dependence of the intensity on the distance between the differential source of radiation and $d\sigma$.

The solution is carried out in spherical co-ordinates, whose origin coincides with $d\sigma$. On the basis of this equation it is possible to determine the exchange of energy between two bordering media and the mean intensity of the dose either in these media or in areas bordering on those where this exchange is taking place.

In evolving equation (1) we made the generally accepted assumption that ionization along the path of an alpha-particle is evenly distributed. We considered it advisable to express the original magnitudes, and the results obtained, in absolute units or their derivatives.

1. EXCHANGE OF ALPHA-RADIATION ENERGIES BETWEEN TWO MEDIA; MEAN INTENSITY OF THE DOSE IN THE BOUNDARY REGIONS OF TWO MEDIA

(*a*) *Flat interface*

Let two media I and II, separated by a flat interface, differ in the concentrations, c_1 and c_2, respectively, of a uniform distribution, in each, of an alpha-radiating substance and in screening power (ρ_1 and ρ_2), and consequently in the passage of alpha-particles (R_1 and R_2).

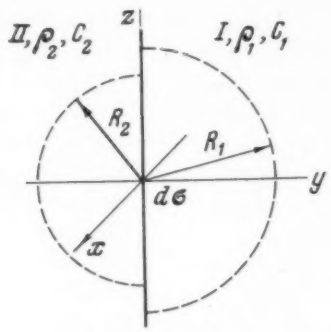

FIG. 1.

Then the flow of energy from medium I into medium II across an element $d\sigma$ of a flat interface (Fig. 1) is determined as follows:

$$dE_1 = \iiint_{V_1} \frac{d\sigma \cos\vartheta}{4\pi r^2} N_1 E_\alpha \left(1 - \frac{r}{R_1}\right) r^2 \sin\vartheta \, dr \, d\vartheta \, d\varphi =$$

$$= \frac{N_1 E_\alpha d\sigma}{4\pi} \left(\int_0^{R_1} dr \int_0^{2\pi} d\varphi \int_0^{\pi/2} \sin\vartheta \cos\vartheta \, d\vartheta - \right.$$

$$\left. - \frac{1}{R_1} \int_0^{R_1} r \, dr \int_0^{2\pi} d\varphi \int_0^{\pi/2} \sin\vartheta \cos\vartheta \, d\vartheta \right) = \frac{N_1 E_\alpha R_1}{8} d\sigma.$$

The flow of energy across a flat interface σ from area I into area II will then equal:

$$E_1 = \iint_\sigma \frac{N E_\alpha R_1}{8} d\sigma = \frac{N_1 E_\alpha R_1 \sigma}{8}.$$

Similarly, for the flow of energy from medium II into medium I across surface σ:

$$E_2 = \frac{N_2 E_\alpha R_2 \sigma}{8}.$$

To determine the total energy absorbed in unit time in the boundary layer of medium I, the thickness of which is determined by the length of the path of the alpha-particles: we must add to the equivalent amount of energy, which would be absorbed in unit time in an identical layer of medium I far from the interface, the energy flowing from medium II, and subtract from it the energy flowing during this time from medium I into medium II. Thus:

$$E_I = E_0 V_1 + E_2 - E_1 = N_1 E_\alpha \dot{R}_1 \sigma + \frac{N_2 E_\alpha R_2 \sigma}{8} - \frac{N_1 E_\alpha R_1 \sigma}{8} =$$
$$= E_\alpha \sigma \left(N_1 R_1 + \frac{N_2 R_2 - N_1 R_1}{8} \right) \text{erg/sec},$$

where $E_0 = N_1 E_\alpha$ is the equivalent amount of energy, absorbed per cm³ of medium I per second; $V_1 = R_1 \sigma$ is the volume of the boundary layer of medium I.

The mean intensity of the dose is determined as follows:

$$\overline{Pd}_1 = \frac{E_1}{V_1} = E_\alpha \left[N_1 + \frac{1}{8} \left(N_2 \frac{R_2}{R_1} - N_1 \right) \right] \text{erg/cm}^3\text{sec}. \tag{2}$$

Similarly:

$$E_{II} = E_\alpha \sigma \left(N_2 R_2 - \frac{N_2 R_2 - N_1 R_1}{8} \right) \text{erg/sec},$$

$$\overline{Pd}_{II} = \frac{E_{II}}{R_2 \sigma} = E_\alpha \left[N_2 - \frac{1}{8} \left(N_2 - N_1 \frac{R_1}{R_2} \right) \right] \text{erg/cm}^3\text{sec}. \tag{3}$$

(*b*) *Spherical interface*

The case of two media separated by a spherical interface will now be considered. Medium I is enclosed in a spherical volume; medium II is outside it. The media possess different screening powers, and different concentrations of alpha-radiating substance.

Let the condition $\infty > a \geq \frac{1}{2} R_1$, where a is the radius of the sphere, be fulfilled.

The flow of energy from medium I into medium II across an element $d\sigma$ of the spherical surface is easily determined if it is divided into two components: one (dE_{11}) —the flow across $d\sigma$ within the limits of the solid angle AOB_2 (Fig. 2); and the

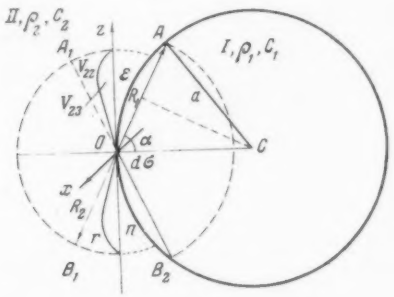

FIG. 2.

second (dE_{12})—the flow of energy from the area between the surface of the cone AOB_2 and the surface of the sphere $A\varepsilon O_n B_2$.

The first component is determined as follows:

$$dE_{11}=\iiint_{V_{11}}\frac{d\sigma\cos\vartheta}{4\pi r^2}N_1E_\alpha\left(1-\frac{r}{R_1}\right)r^2\sin\vartheta\,dr\,d\vartheta\,d\varphi=$$

$$=\frac{N_1E_\alpha}{4\pi}d\sigma\left(\int_0^{R_1}dr\int_0^{\alpha}\sin\vartheta\cos\vartheta\,d\vartheta\int_0^{2\pi}d\varphi-\frac{1}{R_1}\int_0^{R_1}r\,dr\int_0^{\alpha}\sin\vartheta\cos\vartheta\,d\vartheta\int_0^{2\pi}d\varphi\right)=$$

$$=\frac{N_1E_\alpha R_1}{8}d\sigma\,(1-\cos^2\alpha)=\frac{N_1E_\alpha R_1}{8}d\sigma\left(1-\frac{1}{4}\frac{R_1^2}{a^2}\right),$$

$\cos\alpha=\dfrac{R_1}{2a}$, where a is the radius of the sphere (Fig. 2). The second component of the energy flow is arrived at from:

$$dE_{12}=\iiint_{V_{12}}\frac{d\sigma\cos\vartheta}{4\pi r^2}N_1E_\alpha\left(1-\frac{r}{R_1}\right)r^2\sin\vartheta\,dr\,d\vartheta\,d\varphi=$$

$$=\frac{N_1E_\alpha}{4\pi}d\sigma\left(\int_0^{2\pi}d\varphi\int_{\alpha}^{\pi/2}\sin\vartheta\cos\vartheta\int_0^{2a\cos\vartheta}dr\,d\vartheta-\frac{1}{R_1}\int_0^{2\pi}d\varphi\int_{\alpha}^{\pi/2}\sin\vartheta\cos\vartheta\int_0^{2a\cos\vartheta}r\,dr\,d\vartheta\right)=$$

$$=N_1E_\alpha\,d\sigma\left(\frac{1}{3}a\cos^3\alpha-\frac{1}{4}\frac{a^2}{R_1}\cos^4\alpha\right)=\frac{N_1E_\alpha R_1\,d\sigma}{8}\cdot\frac{5}{24}\cdot\frac{R_1^2}{a^2}.$$

The sum of the energy flows across $d\sigma$ from medium I into medium II is

$$dE_1=dE_{11}+dE_{12}=\frac{N_1E_\alpha R_1}{8}d\sigma\left(1-\frac{1}{24}\frac{R_1^2}{a^2}\right)\text{ erg/sec.}\qquad(4)$$

The flow of energy across the total spherical surface is

$$E_1=\iint_{4\pi a^2}dE_1=\frac{\pi N_1E_\alpha Ra^2}{2}\left(1-\frac{1}{24}\frac{R_1^2}{a^2}\right)\text{ erg/sec.}$$

The determination of the flow of energy across $d\sigma$ from medium II into medium I is somewhat more complex.

Its component dE_{21} within the limits of the solid angle $A_1OB_1=AOB_2$, can, evidently, be determined similarly to dE_{11}, and be expressed as follows:

$$dE_{21}=\frac{N_2E_\alpha R_2}{8}d\sigma\left(1-\frac{R_1^2}{4a^2}\right).$$

The flow of energy across $d\sigma$ from the region situated between the surface of the cone A_1OB_1 and the plane xOz, will be only partially absorbed in medium I. At the same time, the energy carried across by alpha-particles, arising in the area between the surface of the cone A_1OB_1 and the surface produced by the rotation of the line described by the equation $r=R_2-r_1(\theta)\dfrac{R_2}{R_1}$, will be completely absorbed in medium I, since the path of the alpha-particles terminates in medium I; those particles, which

arise in the volume enclosed by the surface of rotation r and the plane xOz, will leave only a portion of the energy carried by them across $d\sigma$ into medium I, but it is not difficult to see that this portion will be the same for all alpha-particles of the same direction (Fig. 2).

In the equation given above $r_1(\theta) = 2a \cos \theta$ is the distance in medium I from $d\sigma$ to the surface of the sphere, and $r_1(\theta) \frac{R_2}{R_1}$ is the equivalent distance in medium II.

Considering the above conditions, the flow of energy from a given area of medium II into medium I may be determined as a summation of two terms:

$$dE_{22} = \iiint_{V_{22}} \frac{d\sigma \cos \vartheta}{4\pi r^2} N_2 E_\alpha \left(1 - \frac{r}{R_2}\right) r^2 \sin \vartheta \, dr \, d\vartheta \, d\varphi =$$

$$= \frac{N_2 E_\alpha}{2} d\sigma \left(\int_\alpha^{\pi/2} \sin \vartheta \cos \vartheta \int_{R_2 - r_1(\vartheta)\frac{R_2}{R_1}}^{R_2} dr \, d\vartheta - \frac{1}{R_2} \int_\alpha^{\pi/2} \sin \vartheta \cos \vartheta \int_{R_2 - r_1(\vartheta)\frac{R_2}{R_1}}^{R_2} r \, dr \, d\vartheta \right) =$$

$$= \frac{N_2 E_\alpha}{2} d\sigma \frac{R_2}{R_1^2} \int_\alpha^{\pi/2} \sin \vartheta \cos \vartheta r_1^2(\vartheta) \, d\vartheta = N_2 E_\alpha a^2 \, d\sigma \frac{R_2}{R_1^2} \int_\alpha^{\pi/2} \sin \vartheta \cos^3 \vartheta \, d\vartheta.$$

Integrating, and taking into account that $\cos \alpha = \frac{R_1}{2a}$, we obtain:

$$dE_{22} = \frac{N_1 E_\alpha}{8} d\sigma \cdot \frac{1}{8} \frac{R_2 R_1^2}{a^2},$$

$$dE_{23} = \iiint_{V_{23}} \frac{d\sigma \cos \vartheta}{4\pi r^2} N_2 \frac{E_\alpha}{R_1} r_1(\vartheta) r^2 \sin \vartheta \, dr \, d\vartheta \, d\varphi =$$

$$= \frac{N_2 E_\alpha}{2R_1} d\sigma \int_\alpha^{\pi/2} r_1(\vartheta) \sin \vartheta \cos \vartheta \int_0^{R_2 - r_1(\vartheta)\frac{R_2}{R_1}} dr \, d\vartheta.$$

Successive integration and necessary substitutions give:

$$dE_{23} = \frac{N_2 E_\alpha}{8} d\sigma \frac{1}{12} \frac{R_2 R_1^2}{a^2}.$$

The summated energy flow across $d\sigma$ from medium II into medium I is then obtained

$$dE_2 = dE_{21} + dE_{22} + dE_{23} = \frac{N_2 E_\alpha R_2}{8} d\sigma \left(1 - \frac{1}{24} \frac{R_1^2}{a^2}\right). \tag{5}$$

The flow of energy across the surface of the sphere from medium II into medium I is

$$E_2 = \frac{\pi N_2 E_\alpha R_2 a^2}{2} \left(1 - \frac{1}{24} \frac{R_1^2}{a^2}\right) \text{ erg/sec.}$$

Under the condition that $\infty > a > R_1$ the energy absorbed in a unit of time in a spherical border layer of medium I may then be expressed in the following way:

$$E_1 = N_1 E_\alpha \cdot \frac{4}{3}\pi[a^3 - (a - R_1)^3] + \frac{\pi N_2 E_\alpha R_2 a^2}{2}\left(1 - \frac{1}{24}\frac{R_1^2}{a^2}\right) -$$

$$- \frac{\pi N_1 E_\alpha R_1 a^2}{2}\left(1 - \frac{1}{24}\frac{R_1^2}{a^2}\right) = \pi E_\alpha \left\{N_1 \frac{4}{3}[a^3 - (a - R_1)^3] + \right.$$

$$\left. + (N_2 R_2 - N_1 R_1)\frac{a^2}{2}\left(1 - \frac{1}{24}\frac{R_1^2}{a^2}\right)\right\} \text{ erg/sec}$$

where $V_1 = \frac{4}{3}\pi[a^3 - (a - R_1)^3]$ is the volume of the spherical border layer of medium I,

$$\overline{Pd}_1 = \frac{E_1}{V_1} = E_\alpha\left[N_1 + (N_2 R_2 - N_1 R_1)\frac{3}{8}\frac{a^3}{a^3 - (a - R_1)^3} \times \left(1 - \frac{1}{24}\frac{R_1^2}{a^2}\right)\right] \text{ erg/cm}^3\text{sec.} \tag{6}$$

The energy absorbed in a unit of time in the spherical border layer of medium II is expressed as follows:

$$E_{II} = N_2 E_\alpha \frac{4}{3}\pi[(a + R_2)^3 - a^3] - \frac{\pi N_2 R_2 a^2}{2}\left(1 - \frac{1}{24}\frac{R_1^2}{a^2}\right) +$$

$$+ \frac{\pi N_1 E_\alpha R_1 a^2}{2}\left(1 - \frac{1}{24}\frac{R_1^2}{a^2}\right) =$$

$$= \pi E_\alpha \left\{N_2 \cdot \frac{4}{3}\left[(a + R_2)^3 - a^3\right] - (N_2 R_2 - N_1 R_1)\frac{a^2}{2}\left(1 - \frac{1}{24}\frac{R_1^2}{a^2}\right)\right\} \text{ erg/sec,}$$

$$\overline{Pd}_{II} = \frac{E_{II}}{V_2} = E_\alpha\left[N_2 - (N_2 R_2 - N_1 R_1)\frac{3}{8}\frac{a^3}{(a + R_2)^3 - a^3}\left(1 - \frac{1}{24}\frac{R_1^2}{a^2}\right)\right] \text{ erg/cm}^3\text{sec.} \tag{7}$$

It may be easily seen, that in the limiting case when $a \to \infty$, the expressions (6) and (7) become (2) and (3) for a flat interface.

When $R_1 > a > \frac{1}{2}R_1 V_1 = \frac{4}{3}\pi a^3$, suitable substitutions in expressions for E_1 and Pd_1 finally give:

$$\overline{Pd}_I = E_\alpha\left[N_1 + (N_2 R_2 - N_1 R_1)\frac{3}{8a}\left(1 - \frac{1}{24}\frac{R_1^2}{a^2}\right)\right] \text{ erg/cm}^3\text{sec.} \tag{8}$$

The expression Pd_{II} in this case remains unaltered.

Again, it may be clearly seen that when $a = R_1$, expressions (6) and (8) have coincident values.

When $\frac{1}{2}R_1 > a > 0$, components dE_{II} of the flow of energy from medium I into medium II and dE_{2I} for the flow of energy from medium II into medium I, disappear. The expressions for the remaining components are integrated for θ within the limits from 0 to $\pi/2$. The following expressions are obtained for the mean intensity of the dose:

$$\overline{Pd}_I = E_\alpha\left[N_1 + \left(N_2\frac{R_2}{R_1} - N_1\right)\left(1 - \frac{3}{4}\frac{a}{R_1}\right)\right] \text{ erg/cm}^3\text{sec,} \tag{9}$$

$$\overline{Pd_{II}} = E_\alpha \left[N_2 - \left(N_2 \frac{R_2}{R_1} - N_1 \right) \frac{a^3}{(a+R_2)^3 - a^3} \left(1 - \frac{3}{4} \frac{a}{R_1} \right) \right] \text{erg/cm}^3\text{sec}. \quad (10)$$

It is easy to convince oneself that when $a = \frac{1}{2}R_1$ expressions (8) and (9), (7) and (10), give coincident values. Also when $a \to 0$:

$$\overline{Pd_I} \to N_2 \frac{R_2}{R_1} E_\alpha \text{ erg/cm}^3\text{sec} \qquad \overline{Pd_{II}} \to N_2 E_\alpha \text{ erg/cm}^3\text{sec}.$$

The limiting value for Pd_I is found according to the theory of Gray [4, 5]; the limiting value of Pd_{II} equals the equivalent dose intensity in medium II.

Thus the energy exchange and the mean intensity of the dose in the boundary regions of two media, separated by a spherical interface, have been determined for all values of the radius of the sphere between the limits $0 < a < \infty$.

Fig. 3 gives the curve of the dependence of the mean intensity of the dose on the magnitude $\frac{a}{R_1}$, within the limits $0{\cdot}01 < \frac{a}{R_1} < 100$, for a boundary region of medium I when $N_1 = 0$ (curve $ABCD$).

Section AB of the curve was determined according to the formula (9), section BC according to the formula (8), and CD according to the formula (6).

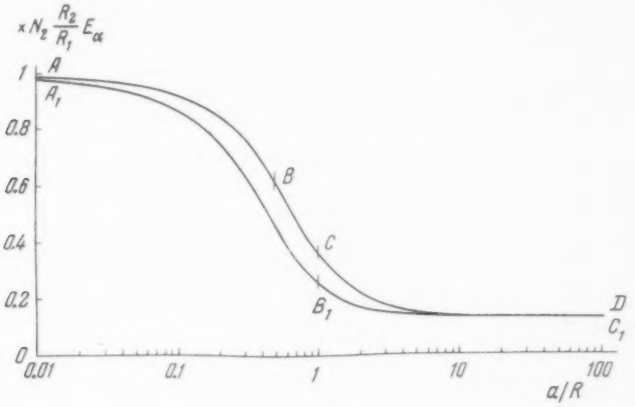

FIG. 3.

(*c*) *Cylindrical interface*

We have not so far been able to find a direct solution of this problem with the characteristics of the two media given above. However, if $a > R_1$, where a is the radius of the cylinder, then it is very simple to obtain an indirect solution which provides sufficiently accurate results.

It may be seen that when $a > R_1$, the energy flow across element $d\sigma$ of a spherical surface (4), (5) differs from those across a similar element of a flat surface by less than 4·2 per cent. Obviously, in the case of a cylindrical interface this difference will be even smaller. Thus the amount of energy flowing across a cylindrical interface may be considered, with small error, to be the same as across a flat interface.

This leads to the following results:

The flow of energy across a part of an infinite cylinder corresponding to the height h, may be determined as follows:

$$E_1 = \iint\limits_{2\pi a h} \frac{N_1 E_\alpha R_1}{8}\, d\sigma = \frac{\pi a h N_1 E_\alpha R_1}{4} \text{ erg/sec}$$

$$E_2 = \iint\limits_{2\pi a h} \frac{N_2 E_\alpha R_2}{8}\, d\sigma = \frac{\pi a h N_2 E_\alpha R_2}{4} \text{ erg/sec.}$$

The energy absorbed in the boundary cylindrical layer of medium I is expressed in the following way:

$$E_1 = N_1 E_\alpha \pi [a^2 - (a - R_1)^2]\, h + \frac{\pi a h N_2 E_\alpha R_2}{4} - \frac{\pi a h N_1 E_\alpha R_1}{4} =$$
$$= \pi h E_\alpha \left\{ N_1 [a^2 - (a - R_1)^2] + (N_2 R_2 - N_1 R_1)\, \frac{a}{4} \right\} \text{ erg/sec.}$$

The mean intensity of the dose in this layer is given by:

$$\overline{Pd}_1 = \frac{E_1}{\pi [a^2 - (a - R_1)^2]\, h} = E_\alpha \left[N_1 + \left(N_2 \frac{R_2}{R_1} - N_1 \right) \frac{a}{4(2a + R_1)} \right] \text{ erg/cm}^3\text{sec}, \qquad (11)$$

and similarly:

$$\overline{Pd}_{II} = E_\alpha \left[N_2 - \left(N_2 - N_1 \frac{R_1}{R_2} \right) \frac{a}{4(2a - R_2)} \right] \text{ erg/cm}^3\text{sec.} \qquad (12)$$

The mean intensity of the dose in medium I within the limits $R_1 \geq a > 0$ may be given approximately by the following expression:

$$\overline{Pd}_1 = E_\alpha \left[N_1 + \left(N_2 \frac{R_2}{R_1} - N_1 \right) \left(1 - 1.4\, \frac{a}{R_1} + 0.65\, \frac{a^2}{R_1^2} \right) \right] \text{ erg/cm}^3\text{sec.} \qquad (13)$$

According to the above expression, when $a \to 0$:

$$\overline{Pd}_1 \to N_2 \frac{R_2}{R_1} E_\alpha \text{ erg/cm}^3\text{sec.}$$

In the interval $R_1 \geq a \geq \frac{1}{2} R_1$, equation (13) gives the value of the mean intensity of the dose with an error not exceeding 4 per cent. This was verified by a supplementary indirect solution, which gives values, within the above limits, agreeing well with those obtained from (13), and which cannot have an error of more than 4 per cent.

This solution was obtained by the same method as (11), but here it was assumed that the difference between the energy flowing across $d\sigma$ of a flat and cylindrical interface was half of this difference between a flat and a spherical interface. In view of the cumbersomeness and the auxiliary nature of this solution, we considered it permissible not to give it here.

It may thus be considered that, within the limits $R \geq a > 0$, the values obtained with the aid of equation (13) are not far removed from the true ones.

Fig. 3 gives a curve of the dependence of the mean intensity of a dose on the

magnitude $\frac{a}{R_1}$ in a boundary layer of medium I in the case of a cylindrical interface (curve $A_1B_1C_1$). N_1 is taken to be equal to zero.

Section A_1B_1 of the curve was determined according to the formula (13), and section B_1C_1 according to the formula (11).

2. THE DISTRIBUTION OF THE INTENSITY OF A DOSE PRODUCED BY ALPHA-RADIATION IN BORDER AREAS OF TWO MEDIA

The absorption of energy, transferred from medium i in an infinitely small volume dA_k of medium k, may be determined on integrating the following equation:

$$dE_i = \iiint_{V_i} \frac{dA_k}{4\pi r^2} N_i \frac{E_\alpha}{R_k} \cdot r^2 \sin\vartheta \, dr \, d\vartheta \, d\varphi, \tag{14}$$

where V_i is the volume of medium i, in which arise the alpha-particles capable of reaching the differential volume dA_k, $\frac{E_\alpha}{R_k}$ describes the ionization per unit length of the path of an alpha-particle in medium k, assuming it to be uniform along the whole path, N_i is the number of alpha-particles produced in a unit of time per unit of volume of medium i.

This equation makes it possible to determine the distribution of the intensity of the dose within the boundary layers of two media.

(*a*) *Flat interface*

Let two media, separated by a flat interface, be characterized by a different concentration of the alpha-radiating substance and by identical screening power.

We are interested in the intensity of the dose at point A of medium I, situated at a distance $l < R$ from the interface between the media (Fig. 4). It is obvious that the

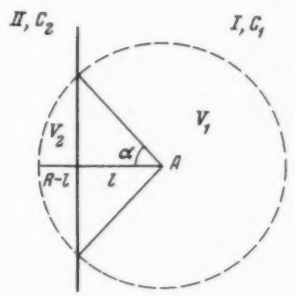

FIG. 4.

intensity of the dose will be produced by alpha-particles, arising in volume V_1 of medium I, and also by alpha-particles arising in volume V_2 of medium II, which comprises a spherical segment of height $R-1$, separated from the sphere of radius R by a flat interface between the media.

If it is assumed that the concentration in medium II is the same as in medium I,

then the energy absorbed in the infinitely small volume dA_1 around point A would be $dE_0 = dA_1 N_1 E_\alpha$ erg/sec.

The additional flow determined by the fact that in volume V_2 of medium II the concentration is not equal to the concentration in medium I may be calculated as follows:

$$dE_2 = \iiint_{V_2} \frac{dA_1}{4\pi r^2}(N_2 - N_1)\frac{E_\alpha}{R} \cdot r^2 \sin\vartheta \, dr \, d\vartheta \, d\varphi =$$

$$= dA_1 \frac{N_2 - N_1}{4\pi R} E_\alpha \int_0^\alpha \sin\vartheta \int_{l/\cos\vartheta}^R dr \, d\vartheta \int_0^{2\pi} d\varphi =$$

$$= dA_1 \frac{N_2 - N_1}{2R} E_\alpha \left(R \int_0^\alpha \sin\vartheta \, d\vartheta - l \int_0^\alpha \operatorname{tg}\vartheta \, d\vartheta\right) =$$

$$= dA_1 \frac{N_2 - N_1}{2R} E_\alpha [R(1 - \cos\alpha) + l \ln\cos\alpha].$$

But, since $\cos\alpha = \frac{l}{R}$ (Fig. 4), consequently

$$dE_2 = dA \frac{N_2 - N_1}{2} E_\alpha \left[1 + \frac{l}{R}\left(\ln\frac{l}{R} - 1\right)\right] \text{ erg/sec.}$$

The total energy, absorbed in dA_1, in a unit of time, may be determined thus:

$$dE_1 = dE_0 + dE_2 = dA_1 E_\alpha \left\{N_1 + \frac{N_2 - N_1}{2}\left[1 + \frac{l}{R}\left(\ln\frac{l}{R} - 1\right)\right]\right\} \text{ erg/sec.}$$

The intensity of the dose at point A is given by:

$$Pd_1 = \frac{dE_1}{dA_1} = E_\alpha \left\{N_1 + \frac{N_2 - N_1}{2}\left[1 + \frac{l}{R}\left(\ln\frac{l}{R} - 1\right)\right]\right\} \text{ erg/cm}^3\text{sec.} \quad (15)$$

If the two media possess different screening powers, then expression (15) becomes

$$Pd_1 = E_\alpha \left\{N_1 + \frac{1}{2}\left(N_2 \frac{R_2}{R_1} - N_1\right)\left[1 + \frac{l}{R_1}\left(\ln\frac{l}{R_1} - 1\right)\right]\right\} \text{ erg/cm}^3\text{sec.} \quad (16)$$

The solution of the equation for medium II is completely symmetrical; hence it may be obtained by a simple substitution of indices in (16), thus:

$$Pd_{II} = E_\alpha \left\{N_2 - \frac{1}{2}\left(N_2 - N_1 \frac{R_1}{R_2}\right)\left[1 + \frac{l}{R_2}\left(\ln\frac{l}{R_2} - 1\right)\right]\right\} \text{ erg/cm}^3\text{sec.} \quad (17)$$

In the particular case when $N_1 = 0$, (16) becomes identical to the formula obtained by Spiers and Munson.

If the distribution of the intensity of a dose within a boundary layer of a medium is known, the mean intensity of the dose may be calculated.

According to the theorem of the mean

$$\int_0^R Pd(l)\,dl = \overline{Pd} \cdot R, \text{ i.e. } \overline{Pd} = \frac{\int_0^R Pd(l)\,dl}{R}.$$

Thus:

$$\overline{Pd_1}R_1 = \int_0^{R_1} Pd_1(l)\,dl = \int_0^{R_1} E_\alpha \left\{N_1 + \frac{1}{2}\left(N_2\frac{R_2}{R_1} - N_1\right)\left[1 + \frac{l}{R_1}\left(\ln\frac{l}{R_1} - 1\right)\right]\right\} dl =$$

$$= E_\alpha\left[N_1\int_0^{R_1} dl + \frac{1}{2}\left(N_2\frac{R_2}{R_1} - N_1\right)\left(\int_0^{R_1} dl + R_1\int_0^{R_1}\frac{l}{R_1}\ln\frac{l}{R_1}\,d\left(\frac{l}{R_1}\right) - \frac{1}{R_1}\int_0^{R_1} l\,dl\right)\right] =$$

$$= R_1 E_\alpha\left[N_1 + \frac{1}{8}\left(N_2\frac{R_2}{R_1} - N_1\right)\right].$$

And finally:

$$\overline{Pd_1} = E_\alpha\left[N_1 + \frac{1}{8}\left(N_2\frac{R_2}{R_1} - N_1\right)\right] \text{ erg/cm}^3\text{sec}.$$

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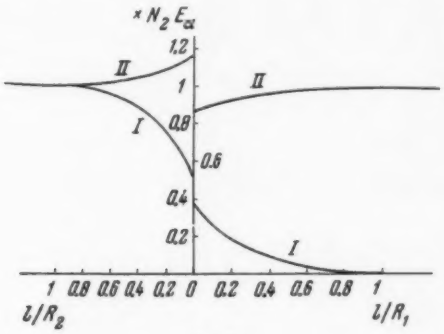

FIG. 5.

Fig. 5 gives the curves of the distribution of the intensity of the dose in boundary layers of two media separated by a flat interface when $N_2 = N_1$ (curve II) and when $N_1 = 0$ (curve I). As the graph shows, the intensity of a dose changes abruptly at

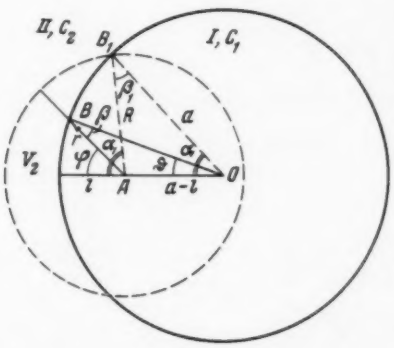

FIG. 6.

the interface of two media—this being conditioned by the abrupt change in screening power on passing from one medium to the other.

This last observation is in complete agreement with the conclusions of Gray [4, 5].

(*b*) *Spherical interface*

Let two media, separated by a spherical interface, possess different concentrations of the radioactive substance and identical screening powers. Medium I is enclosed in a spherical volume, medium II is outside it. Let $\infty > a > \frac{1}{2}R$ where a is the radius of the sphere.

Let us determine the intensity of the dose at point A situated at a distance $l < R$ from the interface (Fig. 6).

Considering first the conditions when $N_1 = 0$; then

$$dE = \iiint_{V_2} \frac{dA_1}{4\pi r^2} N_2 \frac{E_\alpha}{R} \cdot r^2 \sin\varphi \, dr \, d\varphi \, d\varphi' = dA_1 \frac{N_2 E_\alpha}{2R} \int_0^{\alpha} \sin\varphi \int_{r(\varphi)}^{R} dr \, d\varphi =$$

$$= dA_1 \frac{N_2 E_\alpha}{2R} \left[R(1 - \cos\alpha_1) - \int^{\alpha_1} r(\varphi) \sin\varphi d\varphi \right]. \tag{18}$$

To obtain the last integral, it is more convenient to pass from variable φ to variable ϑ.

From triangle AOB it follows:

$$r(\varphi) = r(\vartheta) = \sqrt{a^2 + (a-l)^2 - 2a(a-l)\cos\vartheta}, \tag{19}$$

$$\cos\varphi = \cos(\beta + \vartheta) = \cos\vartheta\cos\beta - \sin\vartheta\sin\beta.$$

According to the theorem of sinuses $\frac{a-l}{\sin\beta} = \frac{r(\vartheta)}{\sin\vartheta}$, from which it follows that $\cos\beta = \frac{a-(a-l)\cos\vartheta}{r(\vartheta)}$; substituting in expression (19):

$$\cos\varphi = a\frac{\cos\vartheta}{r(\vartheta)} - \frac{a-l}{r(\vartheta)}, \tag{20}$$

from which it follows that

$$\cos\alpha_1 = a\frac{\cos\alpha}{r(\alpha)} - \frac{a-l}{r(\alpha)} = \frac{\varphi}{R}\cos\alpha - \frac{a-l}{R}. \tag{21}$$

Differentiating the left-hand and the right-hand sides of (20) we obtain:

$$\sin\varphi d\varphi = a\frac{\sin\vartheta}{r(\vartheta)}d\vartheta + a^2(a-l)\frac{\sin\vartheta\cos\vartheta}{r^3(\vartheta)}d\vartheta - a(a-l)^2\frac{\sin\vartheta}{r^3(\vartheta)}d\vartheta. \tag{22}$$

After substituting expressions (21) and (22) in (18) we obtain:

$$dE = dA_1 \frac{N_2 E_\alpha}{2R}\left[R\left(1 - \frac{a}{R}\cos\alpha + \frac{a-l}{R}\right) - a\int_0^{\alpha}\sin\vartheta\, d\vartheta - \right.$$

$$\left. - a(a-l)^2\int_0^{\alpha}\frac{\sin\vartheta}{r^3(\vartheta)}d\vartheta + a^2(a-l)\int_0^{\alpha}\frac{\sin\vartheta\cos\vartheta}{r^2(\vartheta)}d\vartheta\right].$$

Integrating after uncomplicated but cumbersome transformations the value for dE is obtained from which, after dividing by dA_1, can be determined the intensity of the dose at point A_1, as follows:

$$Pd_l = \frac{N_2 E_\alpha}{2R}\left[(R-l)\left(1+\frac{R+l}{4(a-l)}\right)-\left(l+\frac{l^2}{2(a-l)}\right)\ln\frac{R}{l}\right] \text{ erg/cm}^3\text{sec}.$$

In a general case, when the screening power of the two media is different and N_1 is not 0, the expression for the intensity of the dose at point A becomes:

$$Pd_1 = E_\alpha\left\{N_1+\left(N_2\frac{R_2}{R_1}-N_1\right)\frac{1}{2R_1}\left[(R_1-l)\left(1+\frac{R_1+l}{4(a-l)}\right)-\right.\right.$$
$$\left.\left.-\left(l+\frac{l^2}{2(a+l)}\right)\ln\frac{R_1}{l}\right]\right\} \text{ erg/cm}^3\text{sec}. \qquad (23)$$

When $a \to \infty$ expression (23) coincides with expression (16) for a flat interface. If $\frac{1}{2}R > a > 0$, then expression (18) becomes:

$$dE = dA_1 \frac{N_2 E_\alpha}{2R}\int_0^\pi \sin\varphi \int_{r(\varphi)}^R dr\, d\varphi.$$

After transformations and integration similar to those described above, we obtain for the general case:

$$Pd_1 = E_\alpha\left\{N_1+\left(N_2\frac{R_2}{R_1}-N_1\right)\frac{1}{2R_1}\left[2R_1-a-\right.\right.$$
$$\left.\left.-\left(l+\frac{l^2}{2(a-l)}\right)\ln\frac{2a-l}{l}\right]\right\} \text{ erg/cm}^3\text{sec}. \qquad (24)$$

When $a \to 0$

$$Pd_1 \to N_2\frac{R_2}{R_1}E_\alpha.$$

The expression (24) is applicable within the limits $0 < l < a$. It is somewhat more difficult to calculate the distribution of the intensity of the dose in medium II. However, the procedure is similar to the above, and so only the end results will be presented here.

Within the limits $\infty > a > \frac{1}{2}R_1$

$$Pd_{II} = E_\alpha\left\{N_2-\left(N_2-N_1\frac{R_1}{R_2}\right)\frac{1}{2R_2}\left[(R_2-l)\left(1-\frac{R_2+l}{4(a+l)}\right)-\right.\right.$$
$$\left.\left.-\left(l-\frac{l^2}{2(a+l)}\right)\ln\frac{R_2}{l}\right]\right\} \text{ erg/cm}^3\text{sec}. \qquad (25)$$

Within the limits $\frac{1}{2}R_1 \geqslant a \geqslant 0$

$$Pd_{II} = E_\alpha\left\{N_2-\left(N_2-N_1\frac{R_1}{R_2}\right)\frac{1}{2R_2}\left[a-\right.\right.$$
$$\left.\left.-\frac{1}{2}(2a+l)\frac{l}{a+l}\ln\frac{2a+l}{l}\right]\right\} \text{ erg/cm}^3\text{sec} \qquad (26)$$

The latter expression is applicable when $l < R-2a$. Fig. 7 gives the curves for the distribution of the intensity of the dose between two media, separated by a spherical interface when $N_1 = 0$, and Fig. 8 when $N_2 = 0$.

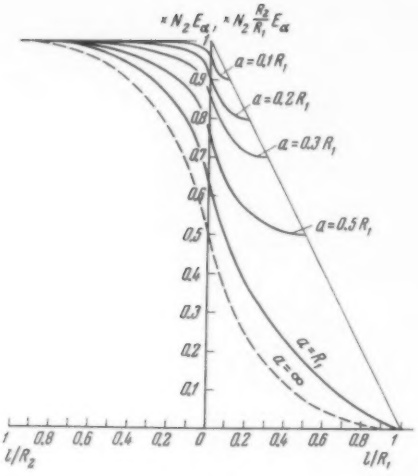

FIG. 7.

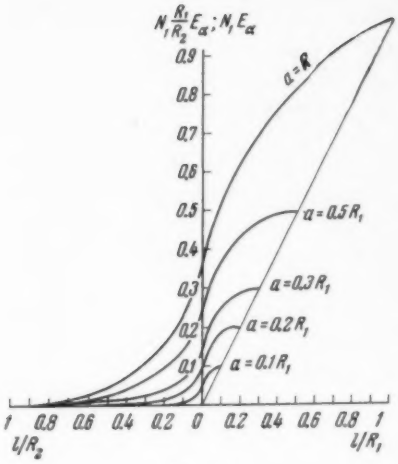

FIG. 8.

3. EXPERIMENTAL VERIFICATION OF THE THEORETICAL CONCLUSIONS

Below are presented the results of an experimental verification of the expression (13) determining the dependence of the mean intensity of the dose on $\frac{a}{R}$ for the case of a medium enclosed within an infinite cylinder. The expression (2), determining the mean intensity of the dose in the case of media separated by a flat interface (cf. Section 1), has also been checked.

It will be recalled that these expressions are as follows:

$$\overline{Pd} = E_\alpha \left[N_1 + \frac{1}{8}\left(N_2 \frac{R_2}{R_1} - N_1 \right) \right] \text{erg/cm}^3\text{sec}, \qquad (2)$$

$$R \geqslant a \geqslant 0,$$

$$\overline{Pd} = E_\alpha \left[N_1 + \left(N_2 \frac{R_2}{R_1} - N_1 \right)\left(1 - 1{,}4\,\frac{a}{R_1} + 0{,}65\,\frac{a^2}{R_1^2} \right) \right] \text{erg/cm}^3\text{sec}. \qquad (27)$$

The choice of a cylindrical interface was due on the one hand to its convenience for experimental investigation, and on the other because the calculations for this interface were made with the greatest number of assumptions and consequently should be the first to receive experimental confirmation. Finally, the results of this experimental verification should apply in large measure to the case of a spherical interface in view of the close connection between the solutions in both these cases. The experiment was carried out as follows:

With the aid of an electrometer (SG-1M) the mean intensity of the dose was determined in the air medium of a cylindrical chamber, whose external electrode was made from paper saturated with the alcohol-soluble uranyl chloride UO_2Cl_2 $(3H_2O)$ (kindly prepared by O. G. Matveev, scientific assistant at the Radiological Department of Ts.N.I.R.R.I.). The paper was 180 μ thick. Thus, conditions for an infinitely-thick alpha-radiation source were fulfilled. To ensure the trapping of ions

produced within the paper cylinder, the paper was stitched with large stitches of copper wire, 0·1 mm in diameter. The screening effect of the wire is negligible. The diameter of the central electrode of the chamber was 1 mm, and this electrode occupied not more than 0·4 per cent of the volume of the chamber, whose diameter was 15·6 mm.

In the experimental determination of the energy absorbed in a section of an infinite cylinder, it is necessary to avoid losses in the energy being determined due to margin effects of the real cylinder. This is easily achieved by extending the chamber on both sides of the radiation-emitting paper cylinder by a length exceeding that of the alpha-particle path. For this purpose, the paper cylinder was placed inside a metal tube. Their heights were 4·5 and 13·5 cm, respectively. The determination of the whole of the effective flow of alpha-particle energy was thus assured. The ratio of this flow to the volume of the radiation-emitting cylinder determines the mean intensity of the dose in an infinite cylinder.

To the external electrode of the chamber was applied a potential of +300 V and +600 V, which was practically sufficient to produce saturation current, as the activity of the alpha-radiation source was of the order of 10^{-2} μc. The ratio $\frac{a}{R}$ was altered by changing the air pressure within the chamber. For this purpose, the metal tube containing the radiation source was placed inside a hermetically sealed emanation chamber of the apparatus SG-1M (Fig. 9). The pressure was measured with a mercury barometer, the atmospheric pressure was determined by an aneroid barometer.

For the determination of the absolute values of ionization currents and of the

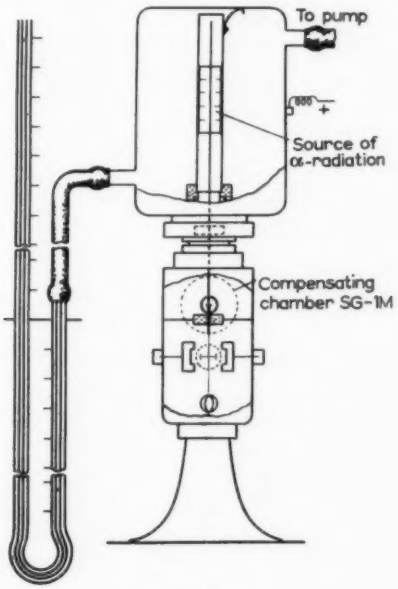

FIG. 9.

mean intensity of the dose, the capacity of the electrometer and the chamber was determined by the method of connecting condensers of small capacity, without noticeable leaks [6].

The measurements of the mean intensity of the dose from a flat source obtained on unrolling the cylindrical radiation source was carried out in a large chamber of the apparatus SG-1M. The approximation of the real conditions of the terminal source to the conditions of an infinite flat interface was performed by a method essentially analogous to that employed in the case of the cylinder. The capacity of the apparatus was determined as above, and also with the aid of a standard of the green oxide of uranium.

The complex alpha-radiation of a natural mixture of uranium isotopes, consisting mainly of alpha-radiations of $^{238}U_I$ with an energy of 4·132 MeV (22 per cent), 4·180 MeV (78 per cent) and of $^{234}U_{II}$ with an energy of 4·716 MeV (26 per cent), 4·765 MeV (74 per cent) was considered, as an approximation, to be equivalent to a monochromatic alpha-radiation with an effective energy of 4·46 MeV, which corresponds to a 3 cm path of alpha-particles in air (760 mm Hg at 15°). Such an approximation cannot introduce too great an error, since the mean intensity of the dose is being determined. The ionizations due to beta- and to gamma-radiations could be disregarded as they were sufficiently low.

The experimental results were compared with the theoretical ones obtained according to the formulae (13) and (2), based on the known concentration of uranium in the paper. The latter was determined by weighing the paper before and after saturation with uranyl chloride after a period of time known to be sufficient for moisture stabilization. Control of the moisture conditions is vitally important. It was carried out by observing the weight of an identical sheet of paper without the uranium salt.

A sheet of paper of an area 24·75 cm² and thickness 0·018 cm contained 0·036 g (±5 per cent) of uranyl chloride. This corresponds to a concentration of the salt of 0·081 g/cm³. A gram-mole of $UO_2Cl_2(3H_2O)$ is equal to 394 g; corresponding to this, the number of molecules of the salt and hence of uranium atoms is $1{\cdot}24\times10^{20}$ per cm³. Since the natural mixture of uranium isotopes contains 99·28 per cent

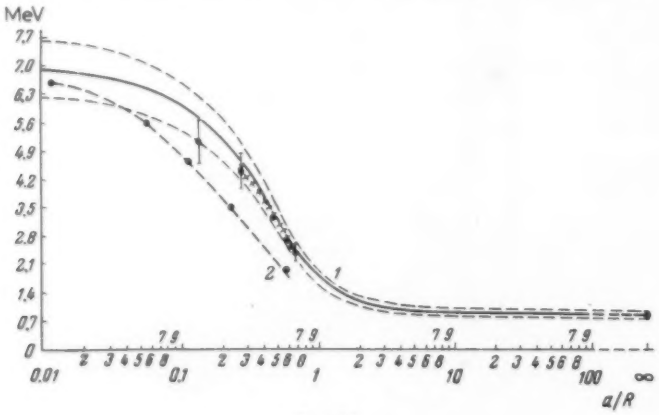

FIG. 10.

$^{238}U_I$ and a quite negligible amount of $^{234}U_{II}$, if the number of atoms of $^{238}U_I$ is taken to be 100 per cent, the number of disintegrations per second of $^{238}U_I$ and an equal number of disintegrations per second of $^{234}U_{II}$ may be calculated with a small —and in this case quite permissible—margin of error. This value equals 605. Correspondingly, the number of alpha-particles with an effective energy of 4·46 MeV is 1210 alpha-particles/cm^3 × sec.

$$NE_\alpha = 5400 \text{ MeV/cm}^3\text{sec}; \; NE_\alpha \frac{R \text{ (paper)}}{R \text{ (air 760 mm Hg)}} = 7{\cdot}0 \text{ MeV/cm}^3\text{sec}.$$

The screening power of 1 g/cm^3 of air is taken to be 1·000 . . . , which corresponds to 0·00129 for air at 760 mm Hg and 15°. The screening power of the paper saturated with uranium salt was found to be equal to 1·0 with a density of 1·00 g/cm^3.

Table 1 gives a comparison of the theoretical and experimental results obtained for the mean intensity of the dose in an aerial medium inside an infinite cylinder under the above-mentioned conditions.

Fig. 10 shows a comparison of the experimental results with the theoretical curve (according to Spiers, curve 2). The theoretical curve was plotted taking into account the error involved in the determination of the concentration of uranium in the paper. Points and crosses on the curves correspond to two series of measurements.

Table 1 and Fig. 10 show that the two series of results agree within the limits of experimental error.

TABLE 1

Pressure (mm Hg)	a/R_{air}	$\overline{Pd}$ (MeV/cm^{3}sec)		$\overline{Pd}$ (MeV/cm^{3}sec, at 760 mm Hg 15°)	
		Theoret.	Exptl.	Theoret.	Exptl.
—	0	—	—	7·0	—
380	0·13	2·88	2·58	5·76	5·16
750	0·26	4·76	4·42	4·82	4·48
850	0·29	5·06	4·79	4·52	4·28
955	0·33	5·33	5·14	4·24	4·09
1060	0·36	5·65	5·48	4·05	3·93
1160	0·40	5·79	5·60	3·79	3·67
1260	0·43	5·99	5·86	3·61	3·53
1360	0·46	6·15	5·94	3·44	3·32
1680	0·57	6·36	6·05	2·88	2·74
1860	0·63	6·41	6·10	2·62	2·49
1960	0·67	6·36	6·16	2·47	2·39

4. CERTAIN QUESTIONS CONCERNING THE ALPHA-RAY DOSES IN SOFT-TISSUE STRUCTURES OF BONES CONTAINING RADIUM

Hoecker and Roofe [7] have published some data on the distribution of radium in the bones of two patients who died of sarcoma involving radium poisoning.

Using these data, Spiers [1] calculated the intensity of the dose in the Haversian canals, lacunae containing osteocytes, and the canaliculi of the bone system. Taking into account that all these elements are of cylindrical shape, Spiers' calculations were based on a substitution for a cylindrical interface of two parallel flat interfaces, separated by a distance equal to the diameter of the cylinder. Having calculated the

distribution of the intensity of the dose in the space between the planes as a sum of the flows across the two flat interfaces, Spiers then obtained the mean of the intensity of the dose, taking into account corrections connected with the difference between the geometry being considered and the cylindrical one. The author does not show how the mean was obtained and how the corrections were introduced.

We have carried out a similar calculation, based on the formula (13), using the data of Hoecker and Roofe [7] and of Spiers [1]. Our results are compared with those of Spiers below.

The following expression forms the basis of Spiers' calculations:

$$\overline{Pd} = NE_{\alpha} \frac{R \text{ (in bone)}}{R \text{ (in } \mu \text{ of tissue)}} \cdot \overline{F}/2 \text{ erg}/\mu^3/\text{day}$$

where $\overline{F}$ is an average function, dependent on geometrical factors.

For the same conditions the expression (13) is transformed thus:

$$\overline{Pd} = NE_{\alpha} \frac{R \text{ (in bone)}}{R \text{ (in } \mu \text{ of tissue)}} \left(1 - 1{\cdot}4 \frac{a}{R \text{ (in } \mu \text{ of tissue)}} + 0{\cdot}65 \frac{a^2}{R^2 \text{ (in } \mu \text{ of tissue)}}\right) \text{erg}/\mu^3/\text{day}. \quad (28)$$

Denoting the factor $\left(1 - 1{\cdot}4 \frac{a}{R \text{ (in } \mu \text{ of tissue)}} + 0{\cdot}65 \frac{a^2}{R^2 \text{ (in } \mu \text{ of tissue)}}\right)$ by $\overline{F}/2$, following Spiers, its value may be compared with the values obtained by Spiers under the conditions studied by him (Table 2). The path of the alpha-particles in soft tissue is taken, after Spiers, to be equal to 44 μ.

TABLE 2

Dimensions of cavity (μ)	$\overline{F}$—average for a cylinder, according to Spiers	$\overline{F}$—average for a cylinder, according to present work
0	2·00	2·00
1 canaliculi	1·89	1·97
5 lacunae containing	1·60	1·84
10 osteocytes	1·35	1·70
20 haversian canals	1·00	1·42
50	0·55	0·82

We used the value of the geometrical coefficient, given by Spiers, for the comparison with our experimental data. The results obtained are shown in Fig. 10 (curve 2). It follows from the figure that the differences between curve 1 and curve 2 for the most part exceed the experimental error. Such a difference in geometrical coefficients leads us to lower levels of the permissible content of radium in the human organism. Table 3 gives these data for a uniform distribution of radium in the bone system. The mean intensity of the dose in the structures studied is higher according to our data than that given by Spiers.

Table 4 gives a comparison of results. Our results differ from those of Spiers and point to a greater intensity of the dose in the structural elements studied.

The author is grateful to the Chief of the Physico-Technical Department of Ts.N.I.R.R.I., V. A. Petrov, for his attention and help in the course of this work.

TABLE 3

Structure	Dimensions of cavity	Limit of permissible concentration in the body (g)	
		According to Spiers	According to present work
		$\cdot 10^{-9}$	$\cdot 10^{-9}$
Haversian canal	50	46·5	31·2
Lacunae containing osteocytes	10	18·9	15·0
	5	16·0	14·0
Canaliculi	1	13·5	13·0

TABLE 4

Structure	Dimensions of cavity	Intensity of dose (rep/day)		
		According to Spiers	According to present work	According to Hoecker and Roofe
Haversian canal	50	6·0	8·9	—
Lacunae containing osteocytes	10	14·9	18·8	23·0
	5	17·6	20·2	
Canaliculi	1	21·2	22·1	—

CONCLUSIONS

(1) A calculation has been carried out of the mean intensity of the dose in boundary regions of two media, separated by a flat, spherical or cylindrical interface, each medium possessing different screening power and a different concentration of the radioactive alpha-particle source.

(2) The distribution of the intensity of the dose within boundary regions of two media, separated by a flat or a spherical interface, has been studied.

(3) Certain conclusions, presented in Section 1, have been experimentally verified. The results obtained have been compared with those of Spiers [1].

Translated by I. GORE

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PROBLEMS OF SPECTRAL ANALYSIS OF STRONG ACOUSTIC STIMULI IN LABORATORY BIOLOGICAL EXPERIMENTS AND UNDER FACTORY CONDITIONS

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(*Received* 4 *November* 1955)

An understanding of the action of the auditory analyser is closely connected with the solution of a number of problems involved in investigation of the laws of the biological action of such physical factors as acoustic stimuli, particularly strong ones.

The solution of these problems has been the concern of physiologists, physicians of various specialities related to the perception of sound, and, more recently, of specialists in industrial health. As a result a number of articles dealing with concepts familiar to the worker in acoustics and to the physicist have appeared on this topic, but, in the authors' opinion, they are useful also for readers who are not specialists in the field of acoustic measurements.

Studies of the effect of acoustic stimuléi on man have, in most cases, been concerned with only one parameter of the acoustic stimulus—the total intensity level [1, 5]. Only during the last years has any attention been given to the frequency composition of the complex sounds acting, since acoustic vibrations with different energy-spectra act differently both on the acoustic receptors and on the central nervous system [3, 6–13].

Many workers have remarked that the action of noise in reducing audibility is greater if the energy is mainly concentrated in the high-frequency region than if most of the energy is in the low-frequency region [7, 8, 14–17].

In the estimation of an acoustic stimulus, account should thus be taken not only of the total intensity level, but also of the other parameters, such as the position of the maximum in the spectral distribution, the limits of frequency to which it corresponds and the sharpness of fall of the magnitude of the spectral level. The data of these parameters give the form of the spectral curve of the stimulus, and can be obtained by spectral analysis.

During the last decade a large number of analysers for simultaneous and particularly for successive analysis have appeared, employing a variety of filters, mixers, and various systems of computation [18–21].

We investigated the different methods of spectral analysis, and compared the results obtained from the analyses performed in these various ways. It was found that it was expedient to supplement one method of analysis (taken as the basic method) by others, in order to give additional information about the physical character of the sounds under investigation.

In biological experiments under laboratory and industrial conditions we used the method of band analysis with octave filters as the basic method. An advantage of

this method is the relative simplicity of the measuring instrument, its reliability under the most difficult conditions, and its adequate accuracy.

This analytical method is suitable for periodic variations, or such as alter slowly by comparison with the rate of operation of the analyser. In most industrial noises the amplitudes of the frequency components, although changing from moment to moment, have the same mean average magnitude over a sufficiently long period of time. Hence industrial noises may be successfully analysed by this method.

When rapidly changing variations have to be analysed, e.g. single impulses, or sounds of short duration, the same method can be used, by first recording them on magnetic tape. Selected sections of the record can then be analysed subsequently.

It is also convenient to use sound-recorders at the same time as the analyser, since the analysis can then be repeated under laboratory conditions, or methods of higher resolution can be used, to obtain a more detailed and accurate structure of the sound vibrations.

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For band analysis we used octave filters with adjacent bands, 16 filters covering the range from 37·5 to 12,800 cycles/sec.

The equipment comprised the pick-up microphone, an amplifier, the octave filters, a self-recorder of the level, and a meter for the amplifier output.

Such a measuring system must be calibrated; i.e. the following parameters must be determined:

(1) The average sensitivity within, and outside, each band.

(2) The effective width of the pass-band for each filter, and the limiting frequencies of these bands, as well as the mean frequency of each band.

(3) The inherent noise level in each band, which defines the lowest level that can be analysed in that particular channel.

(4) The signal level at which distortion appears, which defines the highest signal levels that can be analysed without distortion in any given channel.

The pen-recorder attached to the output may be replaced by a square-law voltmeter. But a self-recorder has the following advantages over the voltmeter: (*a*) the results are automatically recorded in the form of a graph, from which the required values can then be read off with high accuracy when the trace is stationary; (*b*) the tracing itself serves as a documentary record of the measurements; (*c*) analysis is facilitated.

Band analysis gives the total intensity level in each band. When octave filters are used the bands are not of equal width. The direct results from the analyser thus do not give a visual representation of the energy-distribution within the range investigated, and do not directly reflect the subjective impression of the acoustic stimulus, i.e. to calculate the spectral energy levels B may be a function of the frequency f:

$$B_c = F(f).$$

Hence it is necessary, by a simple mathematical treatment of the results of the measurements, to reduce the summated intensity levels in each band to bands of 1 cycle/sec width.

The value of the spectral level (spectral density) of the acoustic stimulus being so obtained over the continuous range, the intensity levels of different spectral regions

can be compared. This would be difficult to do from the intensity levels in the bands covered by the octave filters, since the bands are not of equal width.

We may pass from the levels in the bands to the spectral distribution by means of the expression:

$$B_c = B - 10g\Delta f,$$

where B_c is the spectral intensity of the band in decibels: B is the intensity level of the whole band in decibels, and Δf is the effective width of that particular band in cycles/sec

$$\Delta f = f_2 - f_1,$$

where f_1 and f_2 are the limiting equivalent frequencies of the bands.

The spectral levels in the band are assumed to relate to the geometric mean of the band-frequency f, which is defined by the expression

$$f = \sqrt{f_1 f_2}.$$

As has been pointed out above, in order to observe a more finely subdivided spectrum it is necessary to employ other analytical methods as well, but the method described with a self-recorder makes it possible to draw some conclusions about the nature of the sound vibrations.

Thus, when a self-recorder is used, the fluctuations in the amplitude of the sound within the individual bands, or over the whole range of frequency, can be estimated; but when doing this it is essential to remember that the recorder, having substantial inertia, averages and smoothes out these fluctuations. The number of individual deflections in unit time can be calculated from the records.

In the Acoustic Laboratory of the Biophysical Institute of the Academy of Sciences of U.S.S.R. sound vibrations of complicated composition (noises) of various spectral forms have been used to elucidate the laws of action of strong acoustic stimuli on the auditory analyser, both in man and in animals.

The noises were reproduced in a damped room by means of an electrodynamic reproducer, with different outputs over the sonic range. High-frequency sounds were produced by a group of loudspeakers that reproduced high frequencies well, and the low frequencies were obtained from another group of loudspeakers that were efficient at low frequencies.

The apparatus for the reproduction of sound vibrations comprised the following: noise generator, filters, amplifier, the group of loudspeakers, a voltmeter on the output of the noise-generator, and a voltmeter on the output of the circuit.

The complex sounds produced by the noise-generator had a uniform spectrum over the range 100–10,000 cycles/sec. Inclusion of appropriate filter units enabled one to alter the shape of the spectrum of the acoustic stimulus.

The group of loudspeakers was selected with this end in view. Using an amplifier with a large output, the sound level in the acoustic chamber could be brought up to 120 dB. The range of available sound level was thus very wide, from 70 to 120 dB.

This equipment enabled us to change the form of the spectrum used in the acoustic stimulus in any desired fashion. Four groups of spectral forms were selected.

These four types of stimulus were produced in order to study the reaction of the human auditory analyser as a function of the intensity of the physical stimulus and of its spectral composition. The observations were conducted silently, during the operation of the acoustic stimulus, and after its action.

The four types of spectrum produced with the equipment described above are shown in Fig. 1. The abscissa is the frequency in cycles/sec, while the ordinate is the spectral level in decibels.

Each group of spectra has six curves, which relate to total intensity levels of 70, 80, 90, 100, 110, and 120 dB (see figures on each of the curves).

The spectrum is similar in the range from 300 to 3000 cycles, the curves being almost parallel to the axis of frequency (Fig. 1(*a*)). From 300 cycles downwards, and from 3000 cycles upwards, the curves fall gradually. The rapidity with which the curves fall is 4 dB per octave from 300 to 70 cycles/sec, and from 3000 to 12,000 cycles it is 11 dB per octave, on the average.

Fig. 1(*b*) shows a spectrum in which the energy is predominantly in the low-frequency range, from 70 to 300 cycles/sec. Above 300 cycles the level falls gradually and fairly uniformly. The level falls away at a rate up to 12 dB per octave.

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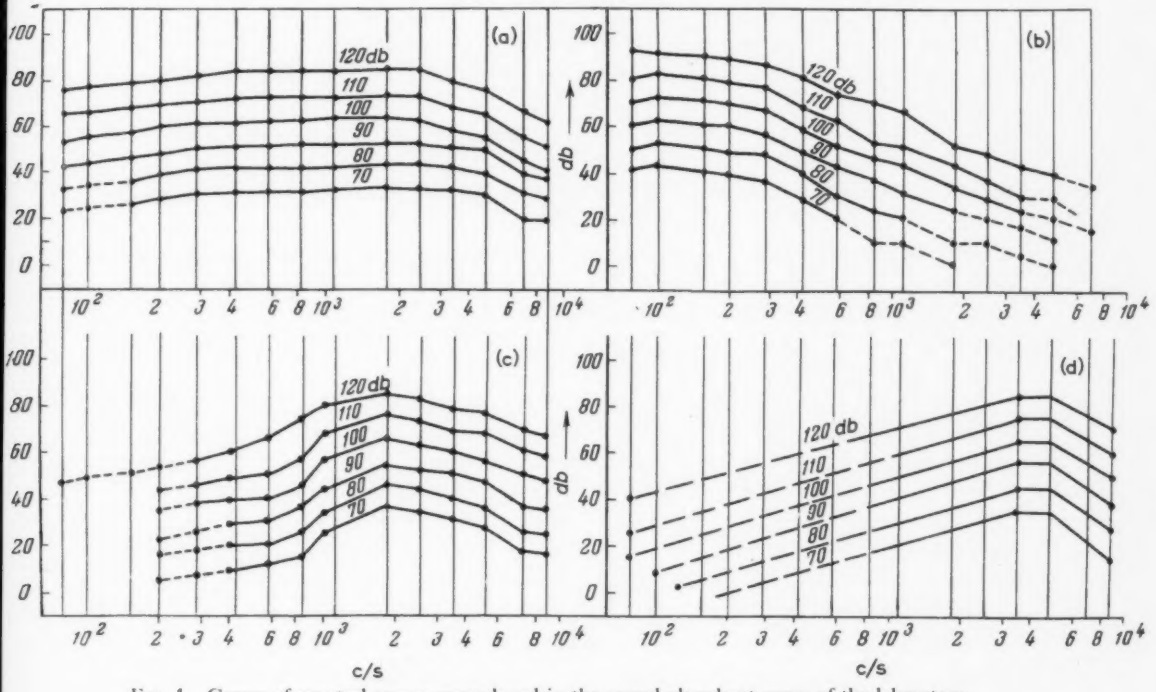

FIG. 1. Group of spectral curves reproduced in the sound-absorbent room of the laboratory.

The spectra shown in Figs. 1(*c*) and (*d*) have maxima in the higher-frequency sonic ranges (1500–2000 and 3500–4000 cycles). The spectral compositions shown in Fig. 1(*c*) fall rapidly on the low-frequency side (12 dB per octave), as compared with Fig. 1(*d*). The converse picture (8 dB per octave) obtains in the high-frequency region. The rate of fall in Fig. 1(*c*) is very much less (7·5 dB per octave) than in Fig. 1(*d*) (15 dB per octave).

The data given show that with one and the same equipment we can produce acoustic stimuli differing considerably in their frequency compositions, but it should

be remarked that the possibilities are not exhausted by the groups of curves given.

Thus in laboratory experiments using such an equipment for the production of various auditory stimuli there lies the possibility of explaining the significance of these or those parameters of auditory stimulus in investigations of their influence on the human organism. The most important of these parameters are the summated level of stimulation intensity, the individual co-ordinates of the maximum of spectral energy and the steepness of fall of the spectral level.

The method of analysis described above may be successfully applied in conditions more complex than those of the laboratory. We have, for instance, used them in investigating acoustic stimuli in metal-working and textile factories.* Under industrial conditions we made wide use of records of the noises on a magnetic tape, as well as determining its spectral composition and total intensity.

The different spectral forms of industrial noises were not so varied as might have been expected at first sight.

Different industrial operations in dissimilar processes may produce spectra that are quite similar to each other. The spectra of industrial noises can therefore be reduced to a limited number of groups.

Thus, when the most important parameters of the industrial noises studied in Moscow factories were analysed, we obtained spectra of four types. These spectra are shown in Fig. 2. The abscissa is frequency in cycles/sec, and the ordinate is the spectral level, relative to the total level, in dB.

Fig. 2(*a*) shows a spectrum frequently encountered. Here the energy is predominantly in the low-frequency region. The spectrum is uniform in the range from 100 to 2000 cycles/sec, the level falling gradually at higher frequencies. This shape of spectrum is characteristic of the textile industry, and for some other noise sources, such as the coal-cutter and pneumatic drills.

The shapes of the spectra shown in Fig. 2(*b*) are encountered in many operations in metal-working. The general noise in the workshops of these undertakings has this form. These spectra show a high spectral density in the region from 400 up to 1500–2000 cycles, with a gradual fall at both ends of the spectral range.

The spectra of Fig. 2(*c*) show maxima at higher frequencies as compared with Fig. 2(*b*) (600–3500 cycles). The rate at which the level falls on the high-frequency side is greater than on the low-frequency side. Spectra of this type were observed in works making ball-bearings.

The spectrum of Fig. 2(*d*) is practically uniform over the range studied, i.e. the energy is uniformly distributed. Spectra of this type were observed in works performing internal grinding and tube-cutting (metal-working industry), and also in the textile industry.

CONCLUSIONS

(1) When considering the effect of acoustic stimuli on the auditory function in man, it is useful to consider the spectra obtained by analysis of the energy-distribution (spectral density) over the frequency range. For this purpose the limits of the fre-

* "Results of some studies of industrial noises in Moscow." Report of the Acoustics Laboratory, Biophysical Institute, Academy of Sciences of U.S.S.R., and of the Hygiene and Epidemological Station, 1954.

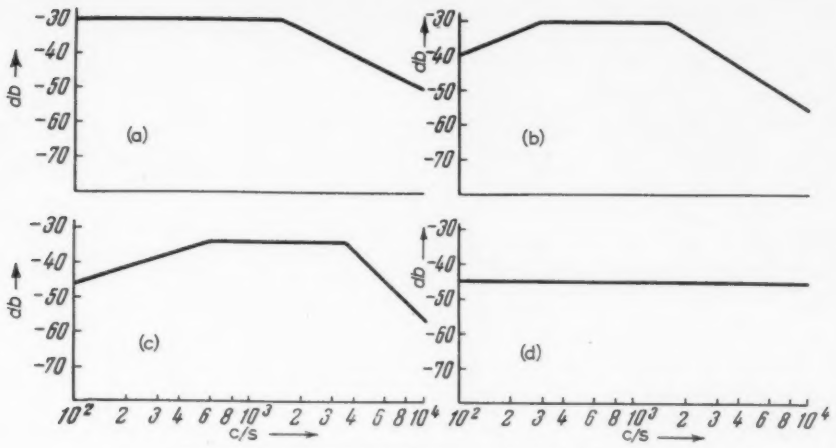

FIG. 2. Idealized types of spectra of industrial noise.

quency region in which most of the energy is concentrated must be determined, as well as the way in which the density changes outside this region. The nature of this latter change can be determined by the steepness of the spectral fall.

(2) Since many industrial operations are characterized by noise-spectra of the same type, there is no need to reproduce the noises of individual industrial processes in the laboratory, it being sufficient to use stimuli with typical spectra.

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A SUBSTITUTION METHOD OF VISUAL COLORIMETRY
(A NEW SYSTEM OF COLORIMETRY FOR INVESTIGATING COLOUR VISION IN MAN)

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(*Received* 18 *May* 1956)

1. Colorimetric investigations of colour vision allow one to study the properties of the primary photoreceptors "in isolation" without interference from the properties of the separate parts in the visual analyser. This is possible because colorimetry is a null method. When two completely indistinguishable fields are set up in the apparatus, the state of the centres has only an inappreciable effect on the result, since the effect on both fields is the same.

In the usual colorimetric method two spatially-distinct fields are compared, which requires that different parts of the retina should be in identical states, and special precautions have to be taken in order to ensure this. It is obviously impossible to carry out an experiment, for instance, if the two parts of the retina are adapted to lights of different colours. Hence the conditions under which colorimetric experiments may be carried out must be exactly stipulated (conditions for colorimetric experiment) [1].

Certain essential requirements must be complied with if any *physically* identical radiations are to be *visually* identical, under the experimental conditions. These conditions are continually violated by the experimental process itself. During practically the whole time of the experiment (save at the instants when the two halves of the field are of identical colour) the observer looks at fields of different colours.

In order to re-create the colorimetric conditions, the experiment must be interrupted at frequent intervals, and the eye adapted to a field of uniform colour.

However, the experiment can be carried out in such a way that the colorimetric conditions are automatically fulfilled. This is done by using fields which are separated not in space, but in time. In this way the same spot on the retina is alternately illuminated by the radiations under comparison. Whereas, in ordinary colorimetry, the radiations are compared as to colour by causing the boundary between the fields to vanish, in the proposed method the two radiations are taken to be of the same colour when the observer sees no change in the field at the moments of substitution of one by the other. It is quite obvious that if any radiation is replaced by a physically identical one (i.e. the colorimetric field simply remains unchanged), the eye of the observer will observe nothing. whatever its state of adaptation. This means, in fact, that in the substitution method of colorimetry, in which one field replaces another, the conditions are automatically fulfilled, without any additional precautions being taken. This eliminates one of the possible sources of error in ordinary colorimetry.

Another phenomenon, which attracted our attention to substitution colorimetry,

and which we first discovered in experiments with frogs, and subsequently detected in man, is that the contrast sensitivity of the eye to changes of light is greatest when the adaptation is to the light under investigation.* On the other hand, the visible difference between two spatially separated fields falls upon adaptation to these fields (this effect is well known). Hence, whereas the usual method of colorimetry reduces the sensitivity of the eye to differences of contrast, in substitution colorimetry the act of measurement increases the contrast sensitivity. So we should expect that the most accurate results would be obtained with the substitution method.

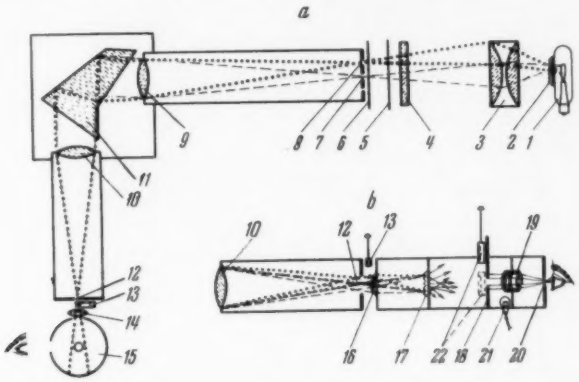

FIG. 1. General layout of the colorimeter, showing the output section, which gives large uniform fields.

(*a*) Plan.

1—projector lamp, 30 volts, 400 watts;
2—ground glass;
3—condenser;
4—filter to remove infra-red radiation (heat absorber);
5—adjustable polaroid;
6—frame with non-adjustable polaroids;
7—slit admitting the light under measurement;
8—one of the basic slits;
9—collimator lens;
10—output lens;
11—prism;
12—exit slit;
13—auxiliary prism used in measuring the wavelength;
14—lens;
15—diffusing sphere.

(*b*) Side view.

16—lens;
17—ground glass;
18—diaphragm;
19—objective;
20—exit diaphragm;
21—small lamp for illuminating diaphragm 18;
22—photocell.

Finally, a third advantage of the method is that it enables one to investigate the colour vision of the retinal periphery.

When adjacent fields are compared, as in the ordinary colorimeter, with a sharp line of separation, attention has to be focused on this line. In the substitution method any point can be fixated at any distance from the experimental field.

All this prompted us to design and construct a colorimeter in which the fields are separated, not in space, but in time, for investigating colour vision in man.

Of course, we do not completely reject the usual colorimetric technique. Our

* It seems to us that this is in good agreement with the results of Rautian and Solovieva [2], who investigated the effect of the surroundings on the acuity of colour-discrimination.

work on the substitution method was undertaken with the object of developing this somewhat original colorimetric principle, so that its positive advantages could be exploited to extend our knowledge of colour vision in man.

2. The principal optical layout of the equipment is shown in Fig. 1. The condenser 3 produces an image of the lamp filament 1 on the system of entrance slits 7 and 8. The ground glass 2 renders the illumination of the entrance slits uniform. By the action of the movable polaroid 5, polarized light falls on the entrance slits. The entrance slits are also covered with polaroid plates, which are orientated in two mutually perpendicular directions (Fig. 2). On rotating polaroid 5 (Fig. 1) through 90° the light ceases to pass through one set of slits, and begins to pass through the other (which are covered by polaroids with their axes perpendicular to those of the first set). The spectral composition of the light emerging from slit 12 depends on the position at any moment of the covered entrance slits.

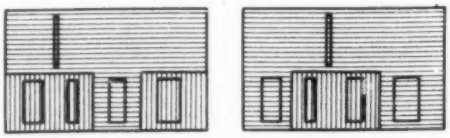

FIG. 2. Examples of the arrangement of polaroids at the entrance slit.

For example, on moving slit *I* (Fig. 3(*a*)) to the right, the wavelength of the light emerging from the apparatus is reduced. Increasing the width of the slit increases the spectral range of the emergent radiation. Hence, by moving the strip which carries the basic slits (Fig. 3(*a*)), we may adjust the basic colours within wide limits for each experiment, according to the problem under study.* The change in the wavelength of the light under investigation was carried out by movement of slit *I* in a horizontal direction. The width of slit *I* was adjusted by the screw 3. The amount of light entering the primary slits was adjusted by the slides 4 (Fig. 3(*a*)). The slides 4 were supported and propelled by rotation of the screws 5, which were joined by flexible cables to knobs adjacent to the observer's hand. The light leaving slit 12 (Fig. 1(*a*)) fell upon the lens 14, which produced an image of the objective 10 on the far side of the sphere 15. The prism 13 was used only to measure the wavelength of the light from the slit *I*. During the time that the subject was making observations it was withdrawn, and lay outside the light-beam. The sphere 15 had three apertures, the centres of which lay on three mutually perpendicular radii. The light entered the sphere through one of them. The light scattered within the sphere entered the eye of the observer through another, while it was passed to a photocell with a known spectral sensitivity through the third.

The construction of the scattering sphere was such as to give a highly uniform visual field even at large angular fields. But the field intensity attained was only a few apostilbs. In some experiments brighter fields were desirable. For this purpose the apparatus was provided with a second modification of the exit section, which made it possible to work with bright fields of small angular dimensions (Fig. 1(*b*)).

* Since the four-dimensional nature of colour distribution in the periphery of the retina had earlier been shown [3], allowance was made in the design of the apparatus for the possibility of working simultaneously with four primary colours.

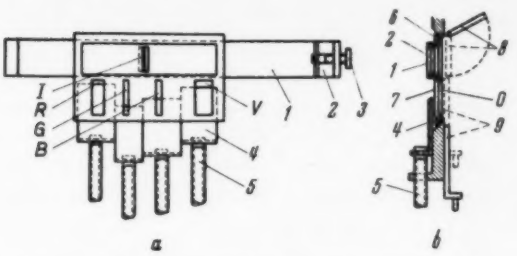

FIG. 3. Entrance slit system of the colorimeter.

(*a*) View from the condenser; (*b*) Side view.

I—slit through which the light being measured passes;
R, *G*, *B*, and *V*—slits through which the red, green, blue, and violet primary radiations pass;
0—basic slit (in profile);
1, 2—strips with knife-edges, which form the slit *I*;
3—screw which drives strip 1 relative to strip 2, thus varying the width of the slit *I*;
4—slide which continuously alters the height of the basic slit;
5—screw which supports and adjusts the slide 4;
6—frame to which the non-adjustable polaroids are attached;
7—polaroid plate;
8—slide which covers the slit *I* while the light is being measured;
9—slide which covers the basic slit while the light is being measured.

The lens 16 produces an image of the objective 10 on the ground-glass screen 17. The lens 19 projects the image of the ground-glass screen 17 on to the observer's pupil. Diaphragm 18 limits the colorimetric field. By altering this diaphragm the angular diameter of the field could be changed. A small lamp, 21, was used when experiments were performed with illuminated surrounds to the test field; this illuminated the whitened surface of the diaphragm. The wavelength of the illuminating beam was measured by inserting the prism 13 into the bundle of rays which thus diverted the light on to a calibrated spectroscope (not shown). The photocell 22 was used to measure the intensity of the light-beam.

3. The actual conduct of an experiment consisted in adjusting the basic slits in such a way that upon substituting a mixture of the radiation under study with some of the primary radiations for the mixture of the remaining primary radiations, the observer noticed no change. The one mixture was substituted for the other by rotating polaroid 5 (Fig. 1(*a*)).

When the requisite degree of opening of the slits had been found, the intensities of both the light under study and the primary radiations were measured. For this purpose the slides 9 and 10 were used (Fig. 2(*b*)), all slits except one being covered. The intensity of the light passing through this unoccluded slit was then measured with the photocell. The measurements were repeated for the other slits.

The variation of the transmission coefficient of the lens 19 with wavelength was determined experimentally. The calculated strength of the light-beam was corrected for this coefficient.

We used barrier-type silver sulphide photocells, type FESS-U2. These were chosen because their spectral sensitivity curves were flat, and the distribution of sensitivity over the surface was uniform. The photocurrent was passed through a

mechanical interrupter (a high-speed relay, driven by a special audio-frequency generator), and then passed to the input of a recording low-frequency amplifier, type 28-IM.

The random errors in measuring the relative energies in spectral regions above 470 mμ were estimated at 3 per cent, for our apparatus; the error reached 5 per cent for measurements below 470 mμ. In order to estimate the possible errors we allowed for the effect of the heights of the basic slits on the light distribution over the photocell surface, the instability and non-linearity of the photocell and amplifier characteristics, the instability of the mechanical interrupter, and the deviation of the 28-IM amplifier parameters from the nominal ones.

In addition to the random errors, systematic errors can also occur, caused by inaccuracy in determining the spectral sensitivity of the photocell. This source of error was estimated at 5 per cent when radiations falling at opposite ends of the visible region were compared. When radiations falling only a short way from one another were compared the possible error was correspondingly less. Errors in the photocell calibration can only affect the absolute determination of the addition curves. When addition curves for several observers are compared, in order to verify colour additivity in the eye receptors, etc., this error is of no importance.

The limits of error within which the wavelengths were measured in our set-up were 1 mμ below 575 mμ, and 2 mμ above 575 mμ.

CONCLUSIONS

The four-colour substitution colorimeter permits the study of colour vision both in the fovea and in the peripheral part of the retina. The experiments may be carried out under the following conditions:

(*a*) over a wide range of intensity (up to 200 apostilbs);
(*b*) with different wavelengths of the primary radiations;
(*c*) with varied angles of visual field;
(*d*) with both dark and light backgrounds to the colorimetric field.

The apparatus is so designed that the intensities of all beams involved in the experiment can be measured.

The light transmitted by any basic slit can be combined directly with the light under investigation. This eliminates the need to use light-dilution techniques. The corresponding errors arising from the dilution are also eliminated.

The substitution method automatically ensures that the conditions for colorimetric experiments are complied with, and eliminates errors due to possible different states of adaptation in different parts of the retina.

The authors wish to express their indebtedness to their fellow-workers in the optical laboratory of the Biophysics Institute; to V. I. Chernishev and V. I. Drozdov, who assisted in the construction of the colorimeter, and to D. A. Shklover, who kindly placed a calibrated photocell at their disposal for calibrating the photocells used in the equipment.

Translated by J. E. S. BRADLEY.

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COLOUR-COMBINATION CURVES FOR NORMAL TRICHROMATS DETERMINED BY DIRECT ENERGY MEASUREMENTS

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(*Received* 18 *May* 1956)

VOL. 2 1957

ALL colorimetric calculations involve the use of normal combination curves, observed with spectra of uniform energy. The combination spectra adopted by the International Illumination Commission (IIC) are based as is well known on the measurements of Wright and Guild with 17 subjects. However, Wright and Guild did not measure the energy-distribution in the spectrum given by their equipment. Instead of this, they recalculated their experimental data, assuming, in essence, the additivity of equivalent intensities. At the present time the available facts show that this assumption is erroneous [1–3]. Hence we might expect that determination of the combination curves, together with direct measurement of the energy-distribution, might result in a change in the form of these curves.

The present article describes the arrangement, technique, and results of a direct determination of combination curves.

The replacement colorimeter is described in the article "A replacement method of visual colorimetry" (this journal, present number, p.) [4]. A variant of the output side was used in order to obtain a field of high intensity. Since foveal vision was being investigated in the present work, only three of the four basic colours available from the instrument were used (610 mμ $< \lambda_1 <$ 690 mμ; 505 mμ $< \lambda_2 <$ 530 mμ; 453 mμ $< \lambda_3 <$ 469 mμ). The angular diameter of the test field was 1·5°.

The experiments were conducted in an illuminated room, in order to reduce the effect of rod vision. The test field had an illuminated surround of approximately the same intensity as that of the field itself. In the breaks between measurements the observer looked at an illuminated white surface. The brightness of the test field was about 100 apostilbs at the middle of the visible spectrum, and about 10 apostilbs at 630 mμ and 450 mμ.

The colorimetric measurements were made as follows: the upper adjustable slit was set to give the required wavelength, which was determined by a calibrated spectroscope. Then the polaroids were adjusted so that the basal illumination, which appears in the equation with a minus sign, was added to the radiation being measured. For this purpose a polaroid plate was arranged in front of the appropriate basal slit with the same orientation as that of the polaroid in front of the upper slit. The polaroids in front of the other slits were arranged with their axes perpendicular to this direction. The observer adjusted the amounts of the primary colours so that there was no visible change in the colorimetric field on turning the movable polaroid.

When visual equality had been obtained in each experiment the relative energies

of each of the primary colours with respect to measured radiation was determined. The measurement was performed with a photocell and amplifier. During the measurement all slits save one were closed, and the difference of the readings given by the amplifier at the two extreme positions of the adjustable polaroid was recorded. This procedure was repeated for all the wavelengths employed.

Colorimetric measurements were made at 10 mμ intervals over the range $680 > \lambda > 430$ mμ. The width of the exit slit on the colorimeter was constant during all the experiments, so the spectral composition of the primary colours remained constant.

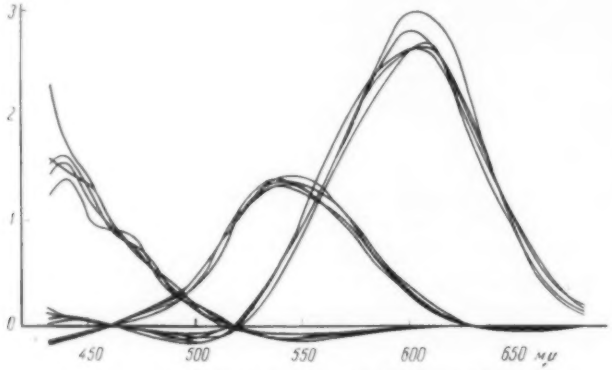

FIG. 1. Combination curves for the five subjects.

Since a photocell of high sensitivity to infra-red was used errors due to the scattering of infra-red light within the apparatus were eliminated by using Shott filters types BG-15 and BG-17 to absorb them.

In order to increase the accuracy with which the energies of the primary colours were measured—particularly when these were small—a set of special calibrated neutral filters were used. During the visual equalization the relevant basic slit was covered with a filter. The filter was removed when the actual energy measurement was being performed. Allowance was then made for the filter factor.

TABLE 1. AVERAGE COMBINATION CURVES FOR THE FIVE OBSERVERS

λ, mμ	*r*	*g*	*b*	λ, mμ	*r*	*g*	*b*
432	0·099	−0·14	1·61	560	1·35	1·19	−0·103
440	0·074	−0·112	1·54	570	1·77	1·00	−0·082
450	0·041	−0·060	1·23	580	2·16	0·76	−0·068
460	0·000	0·000	0·94	590	2·48	0·49	−0·046
470	−0·039	+0·077	0·75	600	2·70	0·31	−0·030
480	−0·078	0·15	0·51	610	2·69	0·16	−0·006
490	−0·103	0·25	0·31	620	2·40	0·051	0·000
500	−0·124	0·43	0·15	630	1·88	−0·003	0·000
510	−0·087	0·72	+0·055	640	1·38	−0·023	0·000
520	0·011	1·06	−0·032	650	0·93	−0·024	0·000
530	0·26	1·27	−0·074	660	0·54	−0·017	0·000
540	0·58	1·36	−0·106	670	0·29	−0·010	0·000
550	0·95	1·31	−0·114	680	0·16	−0·006	0·000

The measurements were carried out with five normal trichromats. The results are given in Fig. 1 and in Table 1.

Since the International Commission's combination curves and our curves are based on different primary colours, they have been recalculated to one and the same basis for the purpose of comparison. It was not convenient to recalculate our curves on the International Commission's basis, since we carried out no measurements at $\lambda = 700$ mμ, while at $\lambda = 435{\cdot}8$ mμ the accuracy was relatively low. Hence the International Commission's combination curves and the mean combination curves for our subjects were recalculated to wavelengths $\lambda_1 = 620$ mμ, $\lambda_2 = 520$ mμ, and $\lambda_3 = 460$ mμ, which were close to the primary colours in our apparatus. The recalculation of our data was performed for the mean figures from the five subjects. The resultant curves are compared in Fig. 2.

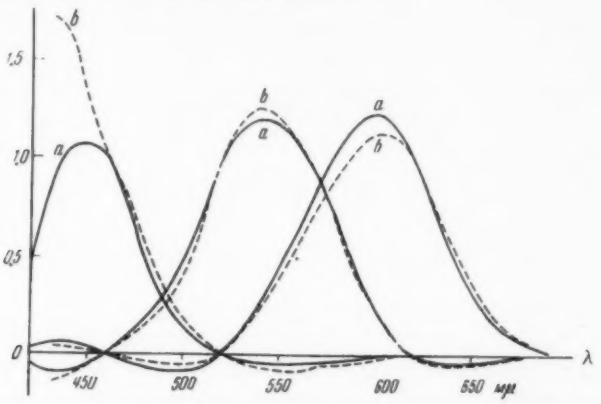

FIG. 2. Average combination curves for our five subjects, and the International Commission's curves, recalculated for 460, 520, and 620 mμ. *a*—IIC curve; *b*—our curves.

The "blue" curves are evidently widely divergent below 460 mμ. The divergence is much greater than the possible experimental error. In order to check this, experiments were carried out in this spectral region with a blue filter (BG-25), which eliminated possible error due to contamination by light of longer wavelengths. These control experiments completely confirmed the difference between the International Commission's curves and ours.

The scatter of the "green" combination curves for different observers was larger than the deviation of the mean from the International Commission's curve, so it is extremely probable that the average curve for many observers would remain close to the International Commission's "green" curve.

For the "red" curves the situation is different. In Fig. 2 it will be seen that there is a systematic though small divergence between our curves and the curve of the International Commission.

The individual differences in the combination curves found in our experiments cannot be explained for certain by random scatter of the experimental results. A direct check on this showed, for instance, that observer B did not make the same settings as observer F, and displaced them in the same direction.

The cause of these individual differences in the combination curves remains to

be sought. It might be supposed that it could be explained by individual peculiarities in the pigmentation of the retina or by coloration of the transparent media of the eye (a general filter in front of all the receptors). In order to check this hypothesis the "red" and "green" combination curves for our subjects were recalculated on a basis such that two of the three primary colours in our arrangement fell in regions of low receptor sensitivity (the recalculations for the "red" and "green" curves were performed on different bases). It is known [5] that after reducing the curves to such a basis the combination curve is close to the sensitivity curves of the corresponding receptors. Fig. 3 shows approximately the curves found for the sensitivities of the "green" and "red" receptors for subjects B and F.

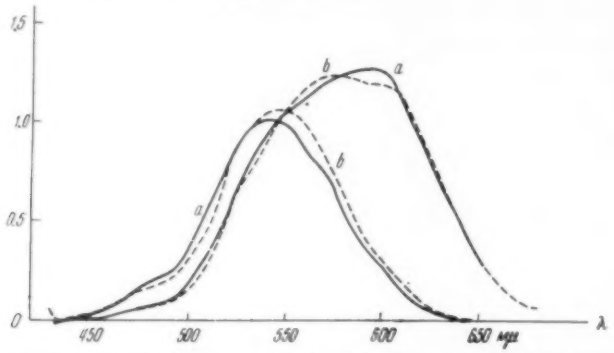

FIG. 3. Approximate sensitivity curves calculated for the "green" and "red" receptors of two subjects: *a*—subject B; *b*—subject F.

The systematic discrepancy between the sensitivities of the "green" receptors is well seen here. There is no such systematic deviation of the sensitivity curves for the "red" receptors. This shows that, in this particular case, the differences between the combination curves cannot be explained by the presence of a filter, since if this were the case there should be differences between the curves for both the "green" and "red" receptors. It is evident that in this particular case the "green" sensitivity curve for one observer is displaced with respect to that for the other.

CONCLUSIONS

To sum up, we may say that our combination curves for equi-energic spectra, which were made by direct (energy) measurements, differ somewhat from the International Illumination Commission's curves, which were obtained by an indirect method. This difference is real, and must be borne in mind, mainly at the ends of the visible spectrum.

The difference of the colorimetric settings for different observers is to be explained at least in some cases by individual properties of the receptors, and not by the presence of a filter in the eye.

Translated by J. E. S. BRADLEY.

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CYBERNETICS IN BIOLOGY

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(*Received* 1 *August* 1956)

VOL. 2 1957

INTRODUCTION

THE general character of cybernetics as a new rapidly developing scientific school has already been given in articles by Sobolev *et al.* [1], Kol'man [2], etc.

It is known that cybernetics began to take shape as an independent field of science after the work of Wiener [3], a prominent mathematician who was also occupied with the design of high-speed automatic control devices for shooting at moving objects. In such arrangements (for example, antiaircraft equipment) the gun continuously "keeps track" of the target (the aeroplane) and must "anticipate" the target so as to make a shot in the direction in which the target is travelling during the time of flight of the projectile. The complexity of the problem consists in the need to determine rapidly the speed of the object, its distance, the time of flight of the projectile (taking into account the influence of wind, temperature, air pressure and other factors), and to find the most probable point where the object will be in the future. Previously, specially skilful and trained people, highly qualified specialists, were required for this, but now all these operations can be carried out automatically by means of so-called tracking devices and high-speed computers.

In designing these devices, and also others (of similar type), in which both hand and automatic control was used there emerged the need to compare the reaction rate of the man and of the electromechanical system, to determine the follow-up errors of the apparatus in both cases, etc. The joint work of engineers, mathematicians, physiologists and psychologists showed that in principle tracking systems, automatic control devices and analogue computers (on the one hand) and of the nervous systems of living organisms (on the other hand) work very similarly, the precise relations of different kinds of movement of material being calculable from the same equations, and the same methods of study being applicable.

At the present time cybernetics is still being established as a science, but its theory and ideas are developing quickly and the range of its practical use is continuously extending. New facts are still being admitted indicating the fertility of using these new ideas in different fields of science and technology.

Two basic lines of development in cybernetics may be differentiated. One is the design of all kinds of computers, automatic controls and communication systems, to some extent reproducing (replacing) certain functional elements in the nervous systems of living organisms; the other is the use of analogue models in biology, physiology and psychology.

The first line (technical cybernetics) has had great successes and has been generally recognized in our country. Soviet specialists are the leaders in the theory of

oscillations, automatic control, information and communication, and the BESM high-speed digital computer is one of the best in the world. But the second line——biological cybernetics—has not developed so extensively because it was not understood and was even opposed by some biologists, and therefore has not made much progress.

This state of affairs may be explained firstly by the "rumpus" raised abroad about the new school (aroused by the desire to extract the greatest profit in the shortest possible time from every sensation), led to the ideas and statements of cybernetics being vulgarized; in numerous articles and publications unfounded statements are expressed, and unfortunate terms are brought in which no doubt repelled biological specialists; secondly, it was due to most biologists and physiologists not having enough training in the exact sciences and unacquainted with the successes of contemporary mathematics, physics and technology, and do not visualize the possibilities which, for example, the use of the well-developed methods and theory of automatic control and information can give for the study of control processes in living organisms.

In this article an attempt is made to survey briefly some prospects of using cybernetics in biology, and ideas and hypotheses are quoted which of course require further consideration and experimental verification. The questions dealt with fall into three main sections: *self-regulation and feedback; analysis and synthesis, time relations; information transfer. Analogues to physiological processes*, which embrace all these sections to some extent, are considered separately.

SELF-REGULATION: FEEDBACK

An automatic follower for guns was mentioned above. Its operating principle is that when the telescope axis (in the optical apparatus) or the receiver antennae (radar units) deviate from the on-target direction, pulses proportional to error appear in the electrical units. After conversion and amplification they operate an electric motor which returns the controlled object (the gun mounting) to the necessary position.

In general, every follower has three main units: (1) a *sensing unit* which reacts immediately the controlled quantity changes (in our example this is the deviation from the on-target direction), and converts these changes to a predetermined signal; (2) a *control apparatus* converting the signals (information) from the detector into directions to the effector mechanism; (3) and *effector unit* which reduces or balances the input change. It contains an effector and a power source.

Feedback is typical of automatic follower systems (Fig. 1, A). Feedback consists in returning part of the controlled quantity voltage output, shaft rotations, displacements, etc., from output to input or to some intermediate point in the loop. If the output and input changes have the same sign they will be amplified and the feedback is *positive*. Systems with large positive feedbacks are unstable, and inherent maintained oscillations may develop when the parameters are appropriate—the systems become self-exciting.

With *negative* feedput the output changes oppose the input changes, and the system becomes self-stabilizing. Negative feedback is used in servomechanisms to ensure zero static error between effector and controlled object and input device. In

continuous followers part of the output (determined by the feedback coefficient) is continuously compared with the input. The *difference* between these, discernible by the error-sensing unit, is also mainly responsible for the follower acting so as to reduce the error. If inertia in the controlled load causes it to overshoot and the error signal sign reverses, the feedback will again cause the follower to return to balance [4].

In biology feedback occurs in any process in which one action is both the consequence and the cause of another. The act of standing may be taken as an example. Equilibrium is assured when the vertical through the centre of gravity falls inside the bearing surface, which is conditioned by impulses from the central nervous system keeping the appropriate muscles in tension. If the centre of gravity begins to move to one side, the muscles on the opposite side will be tensed. Impulses to the central nervous system from the proprioreceptors via the afferent neurones cause a pulse train along the efferent routes to the effectors and to the muscles which will then contract, returning to the previous condition. At the same time less pulses

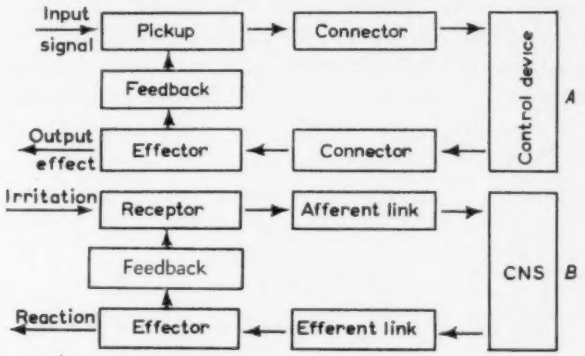

FIG. 1. Feedback circuits in a self-regulator (A) and in a living organism (B).

come from the proprioreceptors and further contraction will cease when the pulses decrease to the original value. But if (due to inertia) the centre of mass overshoots the mean position, pulses of opposite sign flow and the above picture is repeated. Here it is important to note that the extension or contraction of the muscles produced by the efferent pulses causes (via the proprioreceptors) return afferent impulses which themselves change the efferent pulses (Fig. 1, B).

As long ago as 1886 I. M. Sechenov, writing of the role of muscle proprioreceptors in movement co-ordination, said: "A continual experienced stimulation to the nervous centres passes from the moving skin and muscle areas while muscle contraction continues, this nervous stimulation changing in character with movement and thus determining the direction of the motor acts" [5].

Other control processes in the organism involve the same principle (thermostasis, blood pressure constancy, etc.). Without exaggeration one may say that the physiological feedback mechanisms play a very important part in preserving the necessary constancy in the internal environment (homeostasis) when external conditions change [6, 7]. There are reasons for supposing that a similar type of control determines the definite excitability in the central nervous system [8].

Anokhin [9] recently re-emphasized the universality of feedback principles (as

the physiological mechanism of afferent feedback) in *all* reflex acts of the whole organism and their decisive significance in animal adaptation. He considers this is an ". . . afferent signal produced by reflex action", which, like feedback in a servo, is directed from the effector to the controlling organ.

Then the *discrepancy* between the afferent feedback and the "prepared" nervous state in appropriate nervous centres is the cause stimulating adaptation in living organisms (similar to the *error* in the servos above). Thus self-regulation of feedback type is encountered at very different levels in living organisms—from biological cells [10] to the highest nervous activity of animals and, of course, man [11].

ANALYSIS AND SYNTHESIS. TEMPORARY INFORMATION

The circuits and examples of servo and feedback systems considered above are the simplest elements in vital activities. As a matter of fact a living organism is in a state of continuous development, improvement and adjustment to changing conditions in the external environment. Therefore the control and connexion circuits are also continuously changing: the old ones are destroyed and new ones are formed. All this occurs via the analytico-synthetic activity in the central nervous system. We note that the principle of analysis and synthesis is widely used in contemporary computers. The main units in most electronic computers are: input, memory, control, arithmetic and output (Fig. 2).

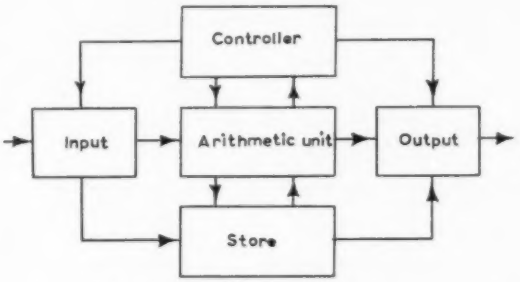

FIG. 2. Block diagram of computer.

Into the input unit come the primary data consisting of coded numbers (or other primary data) and the detailed operating programme. The machine is programmed to complete all operations automatically, in the required order. This programme also predetermines the store of temporary information which will be used in relation to intermediate results which appear during the cycle. The programme is usually fed in as a cipher code (magnetic tapes, perforated paper strips, etc. are used for recording the instructions).

The *arithmetic unit* carries out the instructions—its actions are of the simplest kind—addition and subtraction in the binary system (0 and 1) or the comparison of signals in which the presence of an impulse corresponds to unity and its absence to zero.

The *memory unit* (store) stores the basic codes of numbers and commands and also all the intermediate results used for subsequent operations. Usually two types of memory are distinguished: *working* and *permanent*. The first only stores for a

comparatively short time—intermediate results are preserved until the following operation, and the second is needed throughout the whole operating cycle. The short-term memory uses electronic ring delay circuits and charge-storing beam tubes. The long-term memory is set up on a perforated paper or as a magnetic record on a metal strip, etc.

The *output device* finally works out the results and delivers them in a form suitable for further use. The control head is also informed of the results, comparison with the basic programme being carried out there.

The *control device* ensures that all the actions in the set programme are performed properly, connects and synchronizes the separate units.

The most important feature of the control device (and of the machine as a whole) is that the machine itself can alter the operating sequence when several alternatives are encountered during calculation. This is performed via special "conditional transition" instructions stored in the memory until some signal enters or the results of earlier operations alter.

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Then these commands may alter the sequence of subsequent operations or completely stop the machine (the sequence is predetermined in the general programme).

Thus the computer automatically details its concrete programme, and can allow for the results of all previous operations from rules set up at the start. In other words a principal feature of automatic computers is their self-organizing process determined by the original data (basic rules) supplied, and by the machine design itself. In addition all the operations in a computer consist of performing a long, logical chain, each link having one of two meanings: "yes" or "no" (the simplest analysis), and the subsequent possible choices depend on the results of previous ones (simplest synthesis).

Automatic translation [12] (from English into Russian) may be taken as an example of the use of such machines.

The principles of analysis and synthesis are also used in computers which solve complex logical problems (e.g. play chess), where positions and situations must be considered to find the method of solution [13].

The following remarks may be made when a living organism is compared with such a machine. The main programme exists from birth, being a combination of unconditioned reflexes. However, this programme is more flexible and varied, and it is constantly being perfected and corrected in active life. The ways of performing and correcting this programme are conditioned by central nervous analytico-synthetic activity, by temporary linkage formation mechanisms and by other inherent features of the nerve apparatus as a self-regulating system.

Continuing the analogy, it is interesting to note that some mechanisms in short-term stores which work by recycling pulses round loops (including mechanical) are similar to excitation maintenance processes in cyclic neuron systems. In both cases the excitation may be cut off if a pulse falls in the refractory period of the previous one.

In a machine with a long-term (fixed) memory the information is removed when the programme is finished and a new programme is fed in, no trace of the previous one remaining, unlike in organisms. No action (reaction) disappears entirely in the central nervous system, some trace remaining. In this sense the analogy can be applied only to one machine cycle (programme).

The functional state of an organ or system is known to be very important for the duration of any reflex (including irritation reflexes) (for example, the limb straightens out, if bent, and vice versa, when the skin of a frog's foot is stimulated). Similar mechanisms occur in electronic machines.

Automatic sensitivity control over extremely wide ranges is known in analyser function facts (e.g. eye adaptation). An analogous principle is used in automatic gain control in radio receivers. The input signal may vary by a factor of 10^4 or 10^5 in short-wave reception. Corresponding to this the amplification of the receiver gain changes appropriately to keep the output power constant. An important factor here is to have a certain lag, i.e. the mechanism must react to the intensity integrated over a certain period and not instantaneously. Otherwise the receiver would smooth out the audio signal just as the eye would not distinguish rapid intensity fluctuations.

A follower which predicts the future motion of an object was mentioned above. The trajectory is analysed continuously in such a device, and the most probable future position predicted. A similar analytico-synthetic activity in the cerebral cortex is of great significance in *advance* preparation and mobilization of all the necessary resources for carrying out many complex reflex acts (e.g. jumping on to a moving vehicle, etc.).

INFORMATION TRANSFER

The accurate interaction of all its numerous elements must be a very important condition for the central nervous system to function normally (just as for a computer to work correctly). Excitation and inhibition effects are the main elements in nervous operations in the control of physiological functions, similar to the "on" and "off" elements in an electronic control device.

The neurons in the nervous system are joined by unilaterally conducting synapses forming a complicated conductor system. The conducting directions in the synapses must coincide and the last neuron must be in an appropriate state, if an impulse from an external stimulant is to reach the analyser termination and cause excitation. The pulse summation and excitability increase thus takes place as a result of the complex relations during the transmission of stimuli along different routes—nerve fibres of different length and thickness, with a different number of synapses in which different delays occur. Cybernetics examines similar processes in different feed systems from the information theory standpoint, which is at present the subject of much attention [14]. This theory shows, in particular, that in the transfer of information (signals) along ramified routes with a large number of accidentally combined factors the quantity of information is proportional to the logarithm of the number of factors. As applied to the study of the activity of computers this is some reason for the known Weber-Fechner rule.

The main task of the information theory is the study (with the help of the apparatus of mathematical statistics and the theory of probability) of the rules of conversion and transmission of information in any channels. By information in the wide sense is understood any knowledge of any events, if this knowledge were not known before. It can be any data given through the system of communication or knowledge developed within a certain system. Here are included signals of an outer medium, which operate on living organisms, the instructions sent from the central

nervous system to the effectors, knowledge received by the man during instruction, and so on.

The conception of *information* makes it possible from one point of view to approach the study of the most varied types of information independently of their concrete form and origin. To realize this, conceptions of *quantity* and *size* of the information are introduced. Not all signals contain information but only those which tell us something new about some event, while the information content increases with the unexpectedness of the signal (these problems are dealt with more fully by M. P. Dolukhanov [15]).

The most elementary quantity of information is the answer "yes" or "no" to some question which required only one of two possible answers, both being equally probable or unexpected. This will be called a *binary unit* of information.

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Of course, in many cases which interest physiologists rigid evaluation of the quantity of information is difficult and it is only of importance that the amount of information depends on the unexpectedness of the signal, i.e. it is proportional to the number of possible variants and inversely proportional to the probability of each of them. In information theory it is assumed that any signal may be evaluated in terms of these elementary binary units of information.

It is possible to quote a whole series of examples of the successful solution of some very important problems connected with the theory and practice of communication in telegraphy and radio-broadcasting. It has been shown that existing channels and similar types of communication link were not the best from the point of view of capacity and reliability of transmission, but possible ways of improving them have been indicated. Thus dot-and-dash code used with the alphabet telegraphy has to allow for the different letters being of unequal probability and that the most common ones (in Russian the letters a, o and i), must have shorter symbols. Then the total time spent on transmitting the whole text will be less (or may be sent via a channel of lower pass-band). There is no need to transmit signals containing no information. This principle, of course, is made use of also in ordinary life. For example, if a table is filled in on a form and a number of columns contain the same thing, ditto marks are used or else they are left empty.

Without going into details, we note here an interesting fact from comparative physiology. Certain work [16, 17] shows that the conducting paths in the analysers undergo changes during evolutionary development and specialization, which may be treated from the standpoint of the information theory. Thus, in the higher animals the nerve conductors are differentiated: some fibres, for example of the optic nerve, pass impulses only at the beginning and end of a stimulus, and others pass them only during the stimulus which increases their capacity. Other methods of increasing the transferable information (or reducing the necessary pass-bands), for example, consist of separating the image in television into elements with different rates of sweep, or in using channels with variable pass-bands. A device which reacts to the rate of change of the signal (first derivative) rather than to its size can effect control by these varying parameters (by rate of rise and pass-band). A similarity to these principles may be noted in the way the functional state of the nervous system changes as a function of the signals (information) from external stimuli.

Important conclusions were drawn from the information theory on the question

of receiving weak signals when there are high levels of external and internal noise. Any combination of signals can be regarded as deriving from the interaction of two processes: a regular signal designed to transmit information and a random one caused by randomly varying noise. Signal differentiation and detection are based on the order and not on the amplitude of the process. The "accumulation" of useful signal in the long afterglow cathode-ray tubes, used in radar, illustrates an important principle. It is of great value to apply information theory concepts to the transmission of weak signals along nerve tracks, and to the influence of spontaneous impulses in the nervous system on the accuracy with which individual impulses are transmitted, etc. [18]. It is not accidental that the information transfer system used in nervous systems is more advanced (as regards immunity to interference and signal quality), because pulse-frequency modulation is used instead of amplitude modulation (i.e. the pulse repetition frequency and not the amplitude corresponds to the signal amplitude).

Various automatic control systems are also devices which process information. The essence of the control is that the movements and operations of large masses or large amounts of energy are directed and controlled by the small amounts of energy used in information transfer. The design and operation of any control system is based on this control principle, whether it be an automatic machine or a living organism.

ANALOGOUS TO PHYSIOLOGICAL PROCESSES

The study of complex phenomena using simplified models has long been known in science. However it can be used with particular success in cybernetics because its theory deals only with the quantitative, abstract relations of different types of motion, the nature and type of the processes being irrelevant.

The present revolution in analogue methods was promoted by the development of electronic methods and equipment. Since electronic currents are inertialess, various types of energy can conveniently be transformed into electrical energy, and vice versa, and electrical quantities can be measured accurately, the extremely wide use of electronic analogues in all fields of science and technology is not unexpected.

One possible way of using a simple electromechanical analogy in physiology may be taken from the work of Lebedinskii and Mikhel'son [19]. A special model was used to describe the phenomena and explain the quantitative relations in the visco-elastic properties of skeletal muscle and the changes caused by sympathetic innervation—the model diagram being a circuit containing capacity, inductance and resistance. The frequency of natural oscillation and its dependence on the circuit parameters was determined from standard electrical formulae. Measurements on the torsional oscillations of muscle showed good agreement of theoretical and experimental results.

Analogous to the conduction of excitation impulses along nerve fibres and synapses in the optic nerve have also been devised [20]. An equivalent circuit was constructed from the average characteristics of nerve conductors and nerve centres, and the laws governing the propagation of sinusoidal and rectangular pulses in the circuit were calculated. The hyperbolic relation between the critical frequency of phosphene disappearance (luminous flashings) and the stimulating current observed experimentally was confirmed and explained by the calculation, and effects due to adaptation to this phenomenon were qualitatively evaluated.

On the whole excitation transfer process in nervous systems are very suitable for analogue purposes: firstly, there is a formal analogy: nerve ≡ conductor; secondly, the impulses themselves, both electrical and nervous, have a similar time-sequence. Guliaev's work [21] (comparison of the rhythmical phenomena in nerves and muscles with relaxation oscillations) borders on this.

In some devices it is possible to make a model of what is called in physiology a temporary link. Here we take Pavlov's comparison: "I could have joined my laboratory and residence by direct line and ring when I had to. But now, when I ring the laboratory through the central exchange, this is just the same telephone communication. The difference is only that in one case there is a ready-made conducting route, but in the other a circuit has first to be established" [22]. This analogy could be extended to an automatic telephone station (ATS), in which it would be possible to create conditions for a quicker reply by producing a temporary link of the "change of sensitivity threshold" type.

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It is possible to devise schemes in which an indifferent, "conditional" stimulant (light, sound) is combined with another which is "unconditional" (for example, an electric current) and analogues to phenomena in which a conditioned reflex is accompanied by effects such as "stabilization", "differentiation", "extinction", etc. [23].

The following may be taken as still one more example of analogue principles and of some profound resemblances. When in the technique of control and oscillations (and in many other cases) it is necessary to determine the dynamic behaviour of any electromechanical system, attention is first paid to the *transient response*. The significance of this determination may be explained via the "functional test" concept. Suppose we have a spring balance; to obtain its transient response, it is sufficient to put any weight (within the permitted limit) suddenly on the balance and record the curve of the oscillations excited. If we draw up this curve as a function of time, the shape will be different (Fig. 3) for various balances even if we use the same weight, and dependent on the properties of the system. No inertial system can change to another state instantaneously. The rate of change (slope of the curve) depends on the *mobility* of the system: the new level is determined by the *force* factors. The way the system approaches the new condition plays an important part in the transient response. If the *friction* is weak, the system overshoots the equilibrium position and performs a series of damped oscillations (Fig. 3, curve 1), before finally reaching a

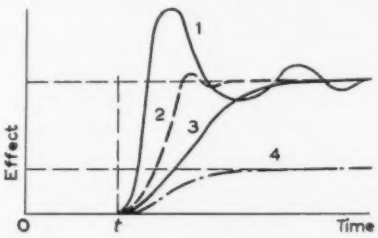

FIG. 3. Transients in different types of system.

steady state (oscillation process). If the daming is increased (by placing the system in a viscous liquid or attaching fins which incur air resistance) the characteristic will be aperiodic (curves 3, 4).

When definite system parameters are used it is possible to obtain a transient response approximating to the ideal, i.e. a rapid rise (curve 2) without subsequent oscillations, which is evidence of balance in the system.

Here we have considered a very elementary example, but just the same method is used in assessing the responses of the most varied self-regulating systems. The transient response is determined by three basic parameters: *power* (gain), *mobility* (or frequency response) and *balance*. If we now go over to analogies, it may be noted that the factors indicated call to mind (speaking carefully) those by which I. P. Pavlov characterized types of nervous system (power, mobility, balance). Then curve (1) would give us the type of a choleric person, (2) sanguine, (3) phlegmatic and (4) melancholic.

Of course, this scheme is very rough, simplified and approximate. However, the papers mentioned above on the analogy between phenomena in nerve-muscle preparations and oscillations in electric circuits give reasons for supposing that the proposed method is quite appropriate for studying similar processes in nerves and muscles, because the transient response may be calculated quite strictly and accurately from the frequency response. The transient response of a nerve-muscle system may be obtained merely by an experimental method, recording, for example, a tendon reflex on an oscillograph.

Of course, to find out the type of nervous system some special (adequate) functional weights should be found and in addition to this it is still necessary to do much work, but in principle the method of investigating the transient response could be used to determine the functional state of the nerve-muscle system just as the measurement of *excitability* and *lability*.

Unfortunately a more detailed argument in support of this statement is outside the scope of this article. In any case it seems to us that the scheme in Fig. 3 *illustrates* much more accurately and graphically the difference of types of nervous systems* than the scheme usually quoted in physiology textbooks. In addition, it widens the horizon of the physiologist and shows that the nerve system, like any self-regulating dynamic system, is characterized by three basic features: *power*, *mobility*, *balance*.

CONCLUSION

Two basic features are characteristic of biological cybernetics as a science: (1) the use of accurate (mathematical) methods of investigating the phenomena, and (2) the development of general principles and analogies in processes of control, self-regulation and contact.

If all the encumbrances and advertisements which have created such a stir abroad are rejected from cybernetics and the two theses mentioned are left, it appears that many of its aspects are quite logical and may be taken advantage of in the development of the science. Moreover, part of the methods of cybernetics such as the modelling of physiological processes and the use of biomechanical analogies in the study of the functions of a living organism have long been known and are successfully used in biology. To confirm these words the expression of A. A. Ukhtomskii may be taken [24]: "Our work will enable us to judge whether we shall be ourselves.

* We note that this scheme can also be obtained by a purely formal method having undertaken the object of expressing on one graph the interrelation of three parameters (power, mobility, balance).

Man once made a machine, and so made it possible to judge how far he himself can be employed as a machine for the production of his work." This statement was developed in more detail by I. M. Sechenov [25]: "The machine idea of the brain in any conditions is a treasure for every naturalist. In his life he has seen so many different apparent machines, beginning from a simple screw to those complex mechanisms which more and more replaced man in the business of physical labour, and he carefully considered these mechanisms so much that if a machine new to him was put before such a naturalist and the interior of it was hidden from his eyes, and only the beginning and end of its activity was shown, then he would work out an approximately correct idea both of the working principle of this machine and of its running."

The present state of technical science (and mainly of electronics) enabled the study of the functional activity of the nervous system to be studied with methods based on electronic high-speed computers.

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It should of course be stated that "rational cybernetics" does not at all identify ideas such as *man*, *animal*, *machine*, but only points out the general principles and schemes of functioning of individual systems which are often characterized by the same terms.

But the main thing is definitely not in the terminology. The question of principle is important: can there in general be common principles in physics and biology? Dialectical materialism teaches us that in spite of the fact that each individual science to a known extent isolates the object of its study from other sciences, these sciences cannot and must not break away from one another, because the different forms of movement of matter which are the objects of study of individual sciences do not in fact exist isolated: they are connected, interweave among themselves and change from one into another.

The problem of the knowledge of the living organism is altogether different from the knowledge of objects of inanimate nature. Of course biology, physiology and psychology have their own specific laws which may supplement the physical laws, be wider or deeper, and may be qualitative and incommensurable, but they cannot go contrary to all the laws of nature, in the expression of which are also the laws of physics, because all the sciences have before them a single (although varied) material world. Here it will be appropriate to quote the words of Academician Lazarev from the article "Laws of physics and laws of biology": "A view of nature binding together the physical and biological sciences gives important results also in the field of a descriptive natural science, and it becomes clear that the laws of physics and biology are not in any way contradictory but that each is only a particular case of rather more general rules which only the limit of present-day knowledge prevents from being discovered at once and to the determination of which all the forces of mankind must be directed" [26].

The process of the knowledge must also be constructed in such a way that on the one hand the general principles are revealed in the phenomena of nature, and on the other hand the specific features of their manifestation in each concrete case are determined. Cybernetics, its methods, built on mathematical logic, studies only general principles, without going into the qualitative characteristics of different phenomena. In this its limit consists, although just this feature allows it to be the same

"universal" (in the conception of its use in other sciences), as mathematics or formal logic. Of course, cybernetics does not at all replace physiology or biology, but it is the connecting link between them and the other accurate natural sciences. It may be suggested that it appears to be very useful for both fields of knowledge.

It should be noted that in the development and attainments of the different natural sciences which have served for a while as a basis for this new science, the labours of our native scientists (A. I. Vyshnegradskii, A. A. Andronov, V. S. Kulebakin, A. N. Kolmogorov—theory of oscillations and automatic control; V. A. Kotel'nikov, A. Ia. Khinchin, A. A. Kharkevich—theory of information, *et al.*) are of outstanding significance. The work of Sechenov, Vvedenskii and, of course, Pavlov were of the greatest importance for the triumph of materialistic ideas and objective methods of investigation in biology. These great scientists always aimed to use in their investigations those resources which gave them the achievements of the accurate natural sciences of that time. They expressed a belief in the fact that such a way seemed fruitful even in the future: "All life from the simplest to the most complex organisms, including of course man, involves coming into equilibrium with the outer medium over a long period, and becoming still more highly complicated. There will come a time, albeit remote, when mathematical analysis having a bearing on the natural-scientific, will embrace with the majestic formulae of equations all these equilibrations including, at last, itself" [27].

For present-day biologists the opportunities have become immeasurably greater, owing to the extraordinary successes of science and technology during recent years. In particular, cybernetics gives new means for accurately investigating and modelling many biological processes. And whether this is called cybernetics or one of the methods of biophysics, we must take advantage of these resources and joint efforts to contribute to the further development of our general science.

Translated by J. GREAVES

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ON THE METHOD OF DETERMINATION OF DIFFERENTIAL SENSITIVITY IN THE DISCRIMINATION OF A SERIES OF SIGNALS

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(*Received* 15 *October* 1956)

The most widespread case of the discrimination of sounds under natural conditions is so-called "absolute" discrimination. When a man has heard some auditory signal he interprets it as one of a series of possible signals already known to him. The discernment of the sounds of speech is an example, where each sound is identified by the hearer as one of the possible phonemes of the language spoken.

The case of discrimination usually investigated in experiments on the physiology of hearing is essentially different, and may be called "relative" discrimination. The subject compares two signals following directly one after the other, and establishes their identity or difference.

The question as to the factors limiting the power of discernment under conditions of "absolute" discrimination is of considerable theoretical and practical interest.

The results of the investigations of Pollack [1] and Garner [2] give evidence that discriminatory ability under conditions of "absolute" discrimination is markedly limited, as compared with that in "relative" discrimination.

Thus, according to these authors' results, a man cannot correctly "absolutely" distinguish more than 16–25 tones differing in frequency and loudness. According to the calculation of Stevens and Davis [3], however, based on results on differential thresholds, the number of distinguishable tones must equal some 340,000.

For the experimental investigation of discriminatory power in "absolute" discrimination it is necessary to have at one's disposal some quantitative measure of auditory discriminatory power that permits of the determination of both absolute and relative discrimination.

The differential threshold is the generally-accepted measure of discrimination,

but all the techniques of determining it known to us [4, 5] necessarily presuppose observance of certain conditions (presentation of a standard, the allowance of only two to three categories of appraisal, a considerable number of gradations of variable sounds) which, naturally, cannot be fulfilled in an experiment on absolute discrimination.

In order to decide whether these conditions are really unavoidable, and whether it is possible to determine the differential threshold (or something equivalent to it) in experiments on absolute discrimination, it is necessary to consider what is, in fact, measured as the threshold in an experiment on the physiology of hearing.

The simplest case logically is the determination of the differential threshold by the method of adjustment. In this case the subject is presented at first with some standard sound with, say, frequency f_k; the subject remembers this, and then, by changing the frequency of the generator, chooses a signal that appears to him to be identical with the standard. As a result of numerous repetitions of this procedure the experimentor obtains a series of the frequency values that were taken by the subject to be identical with the standard. The results are afterwards submitted to statistical treatment: a distribution curve is constructed of the values obtained, the mean value M is found, and the mean square deviation σ from the mean, or the probable deviation ρ, is calculated. It is remarkable how well, as a rule, the distribution curve corresponds with a differential normal distribution curve, with M equal to the standard. As threshold either σ or ρ is taken.

A logical model of this case is furnished by the behaviour of a system (four-pole) in which the stored signal is submitted to random fluctuations, as a result of which the deviations of the signals on the output side from the signals on the input obey a normal distribution. It is evident that the σ of the distribution will be equal to the mean square value of fluctuation in the system. We can therefore define the threshold as the mean square σ or the probable value ρ of the fluctuations in the equivalent four-pole.

Let us consider another way of determining the differential threshold—the so-called *AKh* method (a variant on the traditional "constant" method).

In this case the standard—for example a tone of frequency f_k—is first presented to the subject, then a variable tone which must be determined as higher or lower than the standard.

After many repetitions of the measurements the relation between the percentage of answers, for example, "higher", and the difference of frequency between the variable sound and the standard is plotted as a curve. The difference of frequency corresponding to 75 per cent of answers "higher" is taken as the threshold. Actually, as a rule, the curve obtained corresponds to an exponential curve of normal distribution with $M = f_k$. The threshold is also in this case taken as ρ—the probable deviation. This case can also be represented by a model.

The model consists of the same four-pole with sounds, storing the signal, and an indicator to which the signal from the output of the four-pole (the stored standard subjected to alteration by the fluctuations (x)) and the external signal (the variable tone $f \sim$ *) are applied simultaneously. The indicator gives only two readings:

$$+f\sim > x \text{ and } -f\sim < x.$$

* The symbol $\sim$ denotes the variability.

As the signal on the output of the four-pole fluctuates according to the normal rule it is evident that $P(+)$ as a function of $(f_k - f\sim)$ will be expressed by the integral of a normal distribution curve with parameters $M = f_k$ and (equal to the σ of the fluctuations in the four-pole) and a variable limit of integration

$$f_k - f\sim.$$

When $f_k - f\sim = \rho = 0{\cdot}67449\ \sigma$ $P(+) = 0{\cdot}75$.

In the *AKh* method therefore the threshold may be regarded as the ρ of the fluctuations in a four-pole.

Thus the problem of determining the threshold of discrimination becomes that of the determination, from the subject's answers, of one or other index of the magnitude of the deviation (ρ or σ) in the equivalent four-pole. As it is known that the fluctuations obey a normal distribution the problem is fairly simple, and may be solved also on the basis of the results of experiments on "absolute" discrimination.

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An experiment of this kind involves the presentation to the subject of sounds from a group of signals, previously known to him, equally spaced on the scale of auditory effectiveness. The subject must identify, for example by calling the number by which the sound was previously denoted, each of the sounds of the series when given in random order. The probability of the appearance of each of the sound signals is identical for all the groups. Let us consider the logical scheme of such a case of discrimination.

Following the instructions and preliminary drill the subject memorizes and stores a series or system of signals, to each of which a definite number is assigned. The scale intervals between neighbouring stored signals are of identical magnitude, h, and are termed degrees of quartation in the terminology of the theory of statistics [6–8].

If the position of the system of stored signals on the scale did not change with time we should have a faultless reception, since each transmitted signal would coincide with its corresponding stored signal. It would be otherwise if the system of stored signals fluctuates, or, what comes to the same thing, if, with constancy of the stored signals, the transmitted signals fluctuate.

Suppose the system of stored signals to remain constant, and the transmitted signals to fluctuate, as in this case the treatment will be simpler. It is evident that in the event of fluctuations the transmitted signal will fall on a point of the scale not corresponding to the position of a stored signal of any given number. If the transmitted signal comes to lie nearer to its stored signal than to a neighbouring signal (falls within the limits $\pm h/2$) it will, evidently, be identified correctly. If the given transmitted signal comes to lie nearer to another stored signal it will be taken as the other neighbouring sound. The greater the magnitude of the fluctuations the greater the probability that the distance between the point at which the signal falls and the corresponding stored signal will exceed $h/2$, i.e. that an erroneous answer will be given. Since the fluctuations obey the normal distribution law the frequency of occurrence of the signal at various points of the scale will be given by the formula

$$y = \frac{1}{(\sigma\sqrt{(2\pi)}} \exp\left(-x^2/2\sigma^2\right)$$

where x = scale reading (the reading of the stored signal being taken as 0) and σ the value of the mean square of the deviations in scale units. The probability of a correct

reception of the transmitted signal (P_{tr}), i.e. the probability of its falling on a point of the scale not more remote from the stored signal than $h/2$, will be equal to the integral of this expression between the integration limits of $-h/2$ and $+h/2$

$$P_{tr} = \int_{-h/2}^{+h/2} \frac{1}{\sigma\sqrt{(2\pi)}} \exp(-x^2/2\sigma^2) \quad dx = \frac{2}{\sigma\sqrt{(2\pi)}} \int_{0}^{h/2} \exp(-x_2/2\sigma^2)\, dx$$

Using the substitution $x/\sigma = t$ and replacing $z = h/2$ before integration we obtain an integral of the form

$$\Phi(z) = \frac{2}{\sqrt{(2\pi)}} \int_{0}^{z} \exp(-t^2/2)\, dt$$

—the so-called probability integral, for which tables have been compiled (cf. Bronshtein and Semendiaev [9], Romanovskii [10]).

Knowing the probability of correct reception of a signal (it is determined experimentally from the results of a large number of measurements) we find $z = h/2\sigma$ from the tables if the value of h is given the calculation of σ presents no difficulty.

It should be mentioned that for the signals occupying an extreme position in the series (for example the highest and lowest tones of a given scale) the probability of a correct identification will not coincide with the probability of the transmitted signal being within the limits $\pm h/2$ from its corresponding stored signal. For the elucidation of this let us turn to Fig. 1.

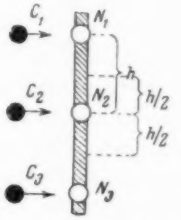

FIG. 1. Scheme of "absolute" discrimination of a series of signals.
C_1, C_2, C_3—transmitted signals; N_1, N_2, N_3—system of stored signals;
h—degree of quartation.

It is evident that signal C_1 will be identified as another sound when it emerges below the limit $h/2$ from its stored signal (N_1). A displacement upwards, no matter how great, does not lead to an error since in this particular example there are no other stored signals above N_1.

Consequently the probability of an erroneous reception of the extreme signals P_{ext} will be half as great as the probability of the occurrence of a signal outside the limits $\pm h/2$ from a stored signal.

$$1 - 2P_{ext} = 2P_{tr} - 1$$

It is interesting to observe that the small probability of error in the perception of extreme sounds has actually been confirmed experimentally in subjects without special training, i.e. in non-musical people (Wedell [11], Pollack [1] and ourselves), which may be regarded as evidence of the adequacy of the model of "absolute" discrimination.

We have calculated the value of σ by the method given from the results of experiments on the "absolute" discrimination of five tones. The degree of quartation varied in the different experiments (for detailed description of the results see the article of Voitinskii in this issue).

In the choice of a unit of measurement for h and σ it was necessary to use one in which the values of σ would be identical at various frequencies. The most recent work on the measurement of threshold discrimination (in particular the work of Harris [12]), carried out with great accuracy on a considerable number of subjects, has shown that the value of the threshold of discrimination ($\Delta f/f$) remains approximately constant over the whole range of frequencies with the exception only of frequencies below 100 c/s (see also the results of Young [13], Koester [14]). We therefore used in our experiments a logarithmic scale of frequencies, choosing as unit of measurement a 1,006-fold increase of frequency, which we provisionally term the "observable difference" (o.d.). This increase is somewhat greater than the

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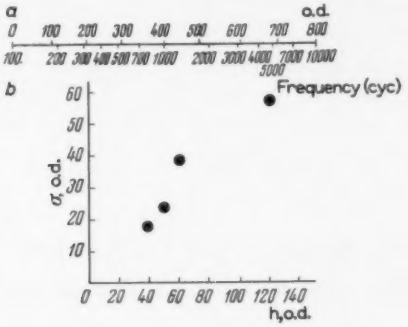

FIG. 2. (a) Scale of frequencies in observable differences. (b) Relation of the value of σ to the value of h. Abscissae values of h ordinates (in observable differences); values of σ in observable differences; data for one subject.

average threshold of discrimination in a sufficiently trained subject. In Fig. 2 the scale of frequencies is given in observable differences; curve b represents the relation between σ and the value of the degree of quartation h as found by experiment. It is seen that σ under the given conditions varies from 20 to 60 o.d., i.e. somewhat exceeds the usual threshold of discrimination. In addition it follows from the curve that by diminishing the steps of the scale σ also diminishes, which is evidence of an improvement of discrimination.

The use of logical models can therefore be useful for the understanding of certain regularities in the variations of the power of discrimination in man.

Translated by T. R. PARSONS

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DIFFERENTIAL SENSITIVITY IN THE "ABSOLUTE" DISCRIMINATION OF A SERIES OF AUDITORY SIGNALS

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(*Received* 15 *October* 1956)

BY "ABSOLUTE" discrimination (judgment or identification) is meant the determination of the pitch or loudness of a sound without comparison with a standard sound. If several sounds are being distinguished in this the hearer has a number of possible answers or choices open to him. The investigation of "absolute" discrimination in the presence of a number of choices is of interest because it is in this way that the recognition of sounds is carried out under natural conditions: having heard a sound we identify it among the whole group of signals conceived by us, and not by comparison with a preceding standard.

In contrast with this there is another possible way of investigating discrimination arising under conditions of the traditional experiment for the determination of the differential threshold. Here there are altogether two or three choices and a standard preceding the variable sound. This considerably facilitates the determination of the variable sound, but introduces an element of artificiality into the discrimination.

The experimental investigation of "absolute" discrimination is often bound up with questions relating to the problem of musical capacities (absolute pitch, musical memory, etc. [1]). In recent years, absolute discrimination has been investigated in

relation to the amount of information that may be received by a human subject from auditory signals.*

In the work of Pollack [5] and Garner [6] it was shown that in the discrimination of sounds differing in pitch or intensity such a quantity of information can be obtained as corresponds to complete discrimination of only 4—5 signals. Recently, Hartman [7] found that a fall of the quantity of information obtained results from an approximation in pitch of the sounds of the series. However, neither this work, nor the investigations of earlier authors, settled a question very essential to the problem of discrimination—namely, the still unelucidated question as to the *threshold difference of frequency* (pitch) involved in "absolute" discrimination of a series of signals.

To judge from the results of Pollack and Garner [5, 6] on the quantity of information, it might be thought that the differential thresholds would be considerably worse than under the conditions of traditional experiment. In addition to this, it was not clear how the values of the thresholds change on approximation in frequency of a series of sounds. Whether they deteriorate in correspondance with diminution of the information received, or whether the changes of the differential thresholds have their own specificity.

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A method of calculation of differential thresholds under conditions of "absolute" discrimination of a series of signals has been suggested by L. A. Chistovich and the author of the present article (cf. the present number). As the magnitude of the differential threshold, σ of the fluctuations of the equivalent four-pole is taken. The magnitude σ can be expressed in units of the scale of pitch appropriate to the sound. As the unit of such a scale an agreed logarithmic measure, called the "perceptible difference" (p.d.), corresponding to an increase of frequency of 1·006—fold, was taken. The magnitude σ can also be calculated from the relation $\Delta f/f$ and Δf for the particular frequency. In this way, it is possible to obtain magnitudes of the absolute and relative differential thresholds, directly comparable with the thresholds established under the conditions of traditional experiment. The aim of the present work is the measurement of the differential thresholds under condition of "absolute" discrimination of five

* For this a calculation from the theory of information is used (Shannon [2], Kharkevich [3], Dolukhanov [4]). According to the postulates of the theory of information, messages containing the information pass from the source to the recipient. The receipt of the information is conceived as the choice of one out of a number of possible messages, and the amount of information transmitted and received can be measured. As unit quantity of information is taken the result of the simplest choice of two possibilities, the probability of each of which is equal to 1/2 (this unit is called "the dual unit" —d.u.). The information transmitted from the source is measured by the natural logarithm of the total number of possible choices presented by the source of information.

Even before the signal is apprehended the recipient knows *a priori* the probability of its arrival. In absolute discrimination of a series of signals, the receipt of each of them is also a choice of one out of a certain group, the recipient knowing beforehand (from the situation or, in an experiment, from an instruction) what signals may arrive.

The receipt of the signal changes the *a priori* probability; in other words, changes the degree of indeterminacy (called by Shannon the entropy), existing before the receipt of the signal, whereby a certain indeterminacy may remain. If we denote the information transmitted from the source as $H(x)$ (entropy of the source), but the information absent from the received message by $Hy(x)$ (conditioned entropy), then the quantity of information actually received is represented by the difference of the entropies: $H(x)—Hy(x)$ [2].

For the calculation of the quantity of information a series of formulae has been suggested [2, 3], one of which is used by us (the technique of the calculation remains unchanged, even in the case of application to the working of an auditory system).

sounds differing in frequency. The measurement makes it possible to answer two questions: it shows what the magnitudes of these thresholds amount to, and how the discriminating activity of the auditory system changes if, from experiment to experiment, the sounds of the series are approximated to one another.

For the comparison of the results with the data in the literature a calculation from the theory of information was employed.*

APPARATUS AND TECHNIQUE

A pure-tone generator type 3G-2A served as source of sound. The sound reached the subject through a relay and attenuator and a dynamic telephone. The subject was in a sound-absorbing room, during the experiment, with the telephone firmly fixed to his ear.

The sound was delivered monoaurally; its duration was 1 sec (regulated by a step relay). The intervals between the sound-signals amounted to 10 sec. The intensity of the sounds adjusted with the help of the attenuator corresponded to 40 dB above the threshold audibility. Communication between the subject and the experimenter outside the chamber was provided by two loudspeakers and an amplifier of type 4-U-12.

The experiment was carried out as follows. A series of tones was given to the subject in arbitrary order, each sound 50 times. To each sound a number had been previously assigned: for this, before the beginning of the experiment, 3–6 min of practice were carried out. In the course of the experiment the subject had to indicate the number of the sound heard. If he made an error, he was corrected by being told the right number.

The differential thresholds were measured with the following intervals of pitch, in other words, steps of scale between the sounds: 120, 60, 40, 20 and 6 pitch differences.

With the step of 120 p.d. the range of frequencies 250–4,000 c/s was combined; with the step of 60 p.d., 500–2,000 c/s; with the step 40 p.d., 630–1,590 c/s; with the step 20 p.d., 800–1,250 c/s; and with the step 6 p.d., 925–1,080 c/s. In these limits the sounds were distributed at equi-logarithmic intervals about the central tone of 1,000 c/s.

The experiments were carried out on 15 subjects possessing normal hearing. They were mostly students, 20–25 years old.

RESULTS AND DISCUSSION

The mean values of the differential thresholds for the five steps of scale are shown in Fig. 1. The magnitude of the thresholds decreases with diminution of the step of the scale.

This phenomenon was observed in all our subjects. The lowering of the differential

* The quantity of information obtained we calculated according to the formula:

$$T_{x,y} = -\Sigma \frac{n_{ij}}{n} \log_2 \frac{n_{i/n}\, n_{j/n}}{nij/n},$$

where n_i = frequency of signal i, n_j = frequency of answer j, n_{ij} = frequency of coincidence of signal i with answer j, n = number of measurements (Miller and Nicely [8]).

The formula gives average values; the unit is dual units per 1 stimulation.

thresholds indicates that on approximating the sound-signals they are more finely differentiated. It may be supposed that this is brought about by the practice arising during the course of the experiments.

The practice consisted of the repeated hearing of the sounds and the correcting of the answers. As Tollack [9] showed, even a simple repetition of the sounds leads to an improvement of the discrimination. In general, it should be said that the power

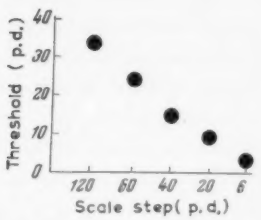

FIG. 1. Magnitudes of differential thresholds according to the results of the group of the subjects. Abscissa: scale step (p.d.); ordinate: values of differential thresholds (p.d.).

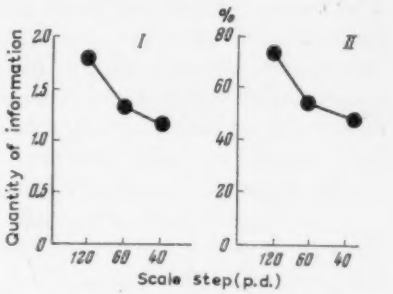

FIG. 2. Curve I: Quantity of information obtained in experiments on "absolute" discrimination of five signals. Abscissa: scale step (p.d.); ordinate: quantity of information (p.d. per stim.).
Curve II: Effectiveness of reception of information. Abscissa: scale step (p.d.); ordinate: percentages. Average values from 6 subjects.

of "absolute" discrimination is much affected by training [1, 10]. We have observed fairly frequently a reduction of the value of the thresholds on repeated measurements in one and the same scale step. There is no doubt that in successive diminution of the step the training leads to a finer discrimination.

The approximation of the sounds of the series leads similarly to the lowering of the amount of information received (Fig. 2), that Hartman [7] found with a somewhat different experimental arrangement. The amount of information received is shown by curve I, which we obtained in experiments on approximation of the sounds, and the relation of the information received to that transmitted, i.e. the efficiency of reception of information, by curve II.

The lowering of the quantity of information received is a completely understandable phenomenon, if it is considered from the point of view of the action of the fluctuations (on fluctuations cf. Chistovich and Voitinskii in the present number).

When the magnitude of the fluctuations is kept constant, but the scale step is diminished, a considerable loss of the quantity of information received must take place, since the fluctuations must "overlap" all the large regions connected with the various signals.

Our empirical results completely correspond to such a suggestion (e.g. at $\sigma = 15$ p.d., Tx,y, amounted to: at a step of 60 p.d., 2·00 d.u. per stim., and at a step of 40 p.d. only 1·74 d.u. per stim.; in the second case the effectiveness of reception fell by 11 per cent in one subject).

Employing the analogy of a coupled system, the considerable loss in the quantity of information received may be regarded as a progressive fall of effectiveness of

reception of the signals, with a constant action of static, the step of quantization being successively diminished.

We have seen, however, that on approximation of the sounds the magnitude of the fluctuations (σ) does not remain constant, but diminishes. It is evident that, on account of this, the quantity of information received is lowered to a smaller extent, in other words, is partially compensated by a rise of the accuracy of discrimination. This compensation is not great. Indeed, it cannot be considerable: it is known from the literature that even prolonged training cannot lead to a large increase of the information received. In the investigation just mentioned, Hartman states that his subject, in the eighth week of training, could not receive a substantially larger quantity of information than in the first days of the experiment (with scale intervals of 50 and 100 units, and the ninth estimates). In both cases the quantity of information received corresponded to the complete discrimination of two to three tones.

On the nature of the magnitudes of the differential thresholds under the conditions of our experiments. A first idea of the threshold can be obtained from a consideration of Fig. 1, in which the average values for a group of subjects are shown.

These results are completely reliable. [Their reliability can be judged by the magnitude of the percentage error of the arithmetical mean (per cent m); for intervals 60, 40 and 20 p.d. the magnitude of m did not exceed 5–9 per cent, for the interval 6 p.d., 1 per cent.]

Our results on the individual subjects naturally differ from the average values given. The individual deviations of the values of the thresholds were the following:

At an interval of 60 p.d. the smallest differential threshold amounted to 16 p.d. (Subj. Ug.), the largest to 34 p.d. (Subj. R-ia). At an interval of 40 p.d. the threshold amounted respectively to 15 p.d. (Subj. Ug. and M-ia) and 34 p.d. (Subj. Tv.). At an interval of 20 p.d.–9 p.d. (4 subjects) and 17 p.d. (Subj. Kom.).

At an interval of 6 p.d. the greatest deviation was equal to 1 p.d. (3 subjects). Thus the results of subjects Ug. (deviation 11 p.d.), R-ia (deviation 7 p.d.) and Kom. (deviation 5·4 p.d.) are the most marked. It should be remarked that the low threshold of subject Ug. was obtained after the carrying out on him of a series of other experiments in the course of which training was involved. On the other hand, the high thresholds of subjects R-ia and Kom. are to a large extent bound up with the fact that these results were obtained in one of the first experiments on each subject. The differential thresholds, determined at different intervals, did not differ markedly from the mean values.

There is no doubt that all our subjects belonged to the wide group of people with normal hearing and the usual attentiveness to sound. They were not persons specially trained as are musicians in the discrimination of sounds, but at the same time they did not belong to a group of people with specially low discriminatory power. But since the values of the thresholds are very much dependent on individual characteristics, among people with "ordinary" discrimination some must be met with who are "typical" for a given interval of deviation. With the help of statistics, it is possible to establish their limits, and, thus, to obtain information regarding the values of the differential thresholds, which, in general, can be measured under our conditions in people of the category mentioned.

Defining the problem statistically, our results must be regarded as small arbitrary

selections relating to a wide series of values of threshold, a series called the general aggregate. What interests us is the average of this general aggregate. The method of determining the limits within which this general average lies is given in many handbooks on the statistics of variations [11].*

The limits between which the general average lies are calculated from our data, as shown in Table 1.

TABLE 1

Interval of scale (p.d.)	Limits (p.d.)
60	21·5–32·5
40	12·9–25·1
20	7·8–15·3
6	5·4–5·8

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From the values given, it is seen that the limits, within which the thresholds vary, are wider the greater the intervals of scale. So far as the interval 120 p.d. is concerned, the values obtained in such an experiment are somewhat complex. The steps between the sounds at this interval are so large that the discrimination proceeds without error, provided the subject is not making the experiment for the first time. If it is the first time, the subject does let errors pass, and, at such an interval, even a small number of errors leads to a substantial increase in the thresholds. We have, therefore, excluded an indication of the average values for this interval. The values given show that the differential thresholds, under condition of "absolute" discrimination of a series of signals, are high, and they considerably exceed the thresholds determined in a traditional experiment. The absolute differential threshold for a single frequency, e.g. 1,000 c/s found in such an experiment, amounts to 3–4 c/s [11, 12]. Under our conditions, the absolute threshold at different intervals varies from 35 to 245 c/s, being thus several tens of times greater than under conditions of traditional experiment.

An analogous picture is also seen when comparing the values of the relative differential thresholds.

According to the results of the authors mentioned, the relation $\Delta f/f$ in the frequency range 250–4,000 c/s does not exceed a few thousandths (it varies, for example, from 0·002 to 0·008), while in our results the relation $\Delta f/f$ may be 100 times larger.

Here we have compared the differential thresholds determined for 5 selections. But a larger number of signals can be "absolutely" discriminated.

* The method of calculation, in a simple form, is as follows: At first, the mean limits of variation of our mean value about the unknown general average is determined. For this, the mean error of the arithmetical mean $(m) = \sigma/\sqrt{n}$, where σ = quadratic mean, n = number of variants. After this, the maximal limits of variation are determined, and the maximal error from the formula:

$$\Delta = \left(3 + \frac{6}{n-4}\right) m$$

Finally, the general mean lying between the limits

$$M-\Delta \text{ and } M+\Delta$$

is calculated, where M = arithmetic mean of the small selection ([13], 323).

What are the values of the differential thresholds with increase of the number of signals to be discriminated? The elucidation of this question is the subject of a separate investigation.

According to our preliminary results, the differential thresholds found with 7 and 9 selections are much higher than those with 5.

In particular, the first requirement for the further characterization of the discrimination is to determine the "minimal" differential thresholds, by which is understood the smallest value of σ found in the course of approximation of the sounds.

Preliminary results from 6 subjects, for whom the "minimal" differential threshold with 5 selections was measured, show that the thresholds here amount to approximately from 2 to 6 p.d.

CONCLUSIONS

(1) Under the conditions of "absolute" discrimination of 5 signals, the differential thresholds for frequency (pitch) are considerably higher than those obtained in classical experiments on the physiology of hearing.

(2) An increase of the number of "absolutely" discriminated signals probably leads to an increase in the values of the differential thresholds.

(3) On approximation of the frequency of the sound-signals, although there occurs a diminution of the quantity of information received, the thresholds are lowered, and the frequency discrimination is finer.

(4) Training, arising in the course of the experiments, is a factor leading to an increase of accuracy of discrimination.

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COLORIMETRIC EXPERIMENTS AS A MEANS OF STUDYING COLOUR VISION: THEIR BASIC REQUIREMENTS

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(*Received* 29 *June* 1956)

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(1) COLORIMETRIC experiments, i.e. tests directed to finding which radiations are visually indistinguishable, yield the most productive means of studying colour vision in man. Almost all the reliable data at present available on the possible mechanisms of colour vision in man were obtained in this way [1, 2]. This is particularly so as regards the light-sensitive receptors in the retina. All the most important practical uses of colour production are based on the data from colorimetric experiments; e.g. colour measurements, tests for and classification of colour vision defects, and theoretical ideas involved in producing coloured images (colour films, colour television), etc.

We might readily dwell on the unique position occupied by colorimetric experiments among methods of studying colour vision. The purpose of any sense organ is to provide varied information about the surrounding objective world. The data given by the eye are solely derived from the fact that objectively different radiations are made visually distinct by causing varied effects at the receptors. Thus all the objectively correct information which the eye is capable of providing is completely defined by the objective differences in radiations which make them distinct from one another. For example, the larger amount of information given by the vision of a trichromat as compared with that available to a dichromat is entirely due to there being certain objectively different radiations which are not distinguishable to the dichromat but are to the trichromat. These objective differences go to make up the extra information given by the third receptor in the trichromat.

The necessary and sufficient conditions to be satisfied by the physical parameters of the radiations if they are to be visually distinguishable in a given system of vision comprise the most important characteristic of the system. This and only this determines the source of the objectively correct information which the eye provides. Colorimetric experiments deal with the question as to which radiations are distinguishable, and which not, in the most direct way. Colorimetric experiments do not answer any other questions, and no other experiments give an exhaustive answer to this most important question for any visual system. This is why colorimetric experiments are of primary theoretical importance. We most certainly do not wish to deny that many other questions, requiring different experiments to answer them, occur when colour vision is studied. But when any visual system is studied it is first necessary to know what kind of objective information it is in general capable of supplying.

Definite conditions must be complied with in colorimetric experiments, and different authors formulate these in various ways. There is not complete clarity in

the literature on what must be fixed and measured when colorimetric experiments are performed. This is the source of many unfortunate misunderstandings.

One of the most striking examples of this is the International Colorimetric standard of 1931. It was at last officially recognized at the Zürich Conference in 1955 [3] that the experimental data on which this standard is based were derived by a defective method since Guild and Wright did not measure the quantitites on which it is based. This example is far from unique.

(2) In this paper we seek to work out the requirements which must be complied with when undertaking colorimetric experiments, and to substantiate them. We first relate this question to the problem of deriving the so-called combination curves, which can give a powerful quantative formulation of the necessary and sufficient conditions for visual distinctness of radiations.*

The aim of a colorimetric experiment which is clearly distinct is to find the radiations which, while different in spectral composition, are visually not distinguishable for a given observer. The experiment consists in making two compared fields indistinguishable, and in determining the objective physical characteristics of the radiations which give visual equality under objectively fixed conditions of observation. The fact of visual equality can, and in principle must, be established objectively (e.g. one must be able to check it objectively when the observer correctly detects the position of the bounding edge in the field, or the moment when one radiation is replaced by the other). But sometimes, to reduce the time required to detect the condition of equality, the observer himself can do this, since practically identical results are obtained in a colorimetric experiment. *A colorimetric experiment should not in any case involve any subjective estimates other than that the fields are visually indistinguishable.*

Since it is not possible to discuss detailed experimental conditions in the abstract, we shall consider which of the conditions has to be kept constant. A general criterion is: *All, and only, those conditions which are required to ensure reproducibility of the experimental results shall be stated.* We shall in future start from this criterion.

The objective factors which are kept constant when carrying out colorimetric experiments can be divided into two groups: (1) the physical features of the radiations under comparison, and (2) all other factors in the objective set-up which can influence the experimental results (size of field, nature of surround, prior adaptation, etc.). These are kept unchanged during a given series of experiments.

(3) As regards the physical parameters of the radiations themselves, one usually says, "the colour of the light is determined by its spectral composition". This statement, which is readily understood, is not, however, sufficiently strong. So we shall replace it by another, which reflection shows only renders the previous one more concrete. "In order that the results of colorimetric experiments shall be reproducible it is necessary and sufficient to fix the spectral compositions of the radiations being compared."†

* These conditions are usually formulated as the conditions that the radiations are visually not distinct, but since these conditions are both necessary and sufficient, they are at the same time the necessary and sufficient conditions for visual distinctness of the radiations.

† We are here speaking of the physical characteristics sufficient to ensure reproducibility, and have in mind only those which affect the radiations compared. In addition it is necessary, as has already been stated, to fix definite conditions under which the fields are compared. These conditions will be considered below.

This discussion would not give rise to any doubts were it not that the question of whether to fix the absolute or the relative spectral compositions of the radiations remains. This question requires careful attention, since neglect to pose it has led to much misunderstanding and to direct errors. The main source of misunderstanding is evidently the ambiguity of using the terms "absolute" and "relative" spectral composition, on the one hand, and "absolute" and "relative" measurements on the other. By the relative spectral composition one means the ratio of the energies present in different wavelength ranges in a single radiation. It is clearly insufficient for ensuring reproducibility to fix this characteristic of the radiations used in the colorimetric experiment only. Radiations which are identical in relative spectral composition are visually the same only when the radiation fluxes in the two fields compared are the same. In this sense we have the right to say that the absolute and not merely the relative spectral composition must be fixed in order to ensure reproducibility; or, more exactly, the absolute spectral distribution of radiant energy (energy per unit time per unit area of the retina).

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Does this imply that the spectral distribution parameters must be measured in absolute CGS units? We shall show that this is far from being always so.

One of the consequences of Grassman's additivity law, which so far has always been considered correct, is: "If the energy fluxes in two visually identical fields change by the same factor without change in the relative spectral compositions, then visual equality is maintained."

According to this, the conditions of visual identity have the same numerical expression in all systems of units, provided that these units remain the same for all the radiations compared. We may thus certainly say that only the ratio of the energy fluxes is important and not their absolute values. But this does not mean that it is sufficient to know only the relative spectral composition of the radiation, since we are concerned not with the ratio of energies of different wavelengths within the same radiation, but with the ratio of the total energies in the different radiations. To find this latter for each of the radiations separately the absolute energy fluxes must be found. This may be done in any system of units which is the same for both fields, since all values change equally on passing from one system of units to another.

This discussion shows why in colorimetric work it is not usually necessary to refer the data to an absolute system of units, although the absolute and not the relative spectral composition of the radiation is important. Hence the possibility of avoiding transferring the experimental data to an absolute system of units is due to the tacit assumption that Grassman's law is correct for the colour vision system used. In any other case we should certainly have to express the absolute spectral composition of the radiation in absolute units as well as taking it into account. We must point out that when combination curves are derived this law must certainly be taken as correct, otherwise the combination curves are not in general completely defined.

Summing up, we may say: in all colorimetric experiments it is necessary and sufficient to fix the absolute spectral distributions of radiant energy for all radiations compared in order to ensure that the results be reproducible. When Grassman's law can be safely assumed it is quite permissible to take the measurements in arbitrary units which are the same for all the radiations and which are unrelated to the CGS system.

The discussion implies in particular that with monochromatic radiations it is

necessary not only to fix the wavelengths, but also to fix the energy supplied to the field of view.

The combination curves taken by Guild and Wright [4] have already been referred to as being erroneous, since they were not derived from measurements of the energy fluxes in the fields on the equipment. Otherwise put, the objective arrangements made in the experiment are known to be inadequate. In addition Guild and Wright used subjective comparison ("by brightness alone") on radiations known to be visually distinct, and recomputed the data using the so-called visibility curves. The fact that this method is unsound is now realized, since there are important deviations from additivity in heterochromatic comparison. In particular our analysis shows that this was not the only fault, there being errors of principle in setting about the problem. After visual equality has been decided on we only need to know the purely physical characteristics of the radiations, and, as in any other purely physical investigation, it is not permissible to use additional subjective adjustments for these purposes. In spectrophotometric experiments, for example, it is most improbable that anyone would define the energy of radiations of different wavelengths using heterochromatic comparisons and calculations from visibility curves.

(4) The phenomena of successive and simultaneous contrast, colour constancy, etc., show that the visual estimation of field colour equality depends, in general, not only on the radiations compared but also on their spatial and temporal relations. Definite conditions must therefore be observed in order to obtain reproducible results. These are usually stated either in a general form, such as "the retinal state must be the same for the two fields which are compared", or in a more concrete form, in which the field size, nature of surround, preliminary adaptation, etc. are stated.

The requirement that the retinal areas used must be in the same state is of obvious meaning, but it is quite obscure how to fulfil this requirement and check that it is fulfilled in any concrete experiment. Statements of the field size, nature of surround, etc. do not make certain whether all such statements are necessary and whether any of them is sufficient. Various workers make different statements on this. We therefore propose a new and, it is to be hoped, a fairly general definition of the colorimetric conditions for comparison.

In our statement of the problem the aim is to ensure that the results of experiment will be reproducible when the radiations used remain unchanged. For this purpose limitations on the experimental conditions are introduced. The following definition is derived from this: *Colorimetric conditions of comparison. The conditions of comparison are termed colorimetric if two physically identical radiations are visually identical.*

It follows directly from this definition that the conditions of comparison are termed colorimetric if, and only if, the result of visual comparison of any radiations depends solely on the physical parameters of the radiations and on nothing else (such as, in the case of man, their spectral composition). This ensures that the conditions formulated as both necessary and sufficient. On the other hand the definition states directly how observance of these conditions may be checked in any concrete experiment.*

* It is curious that although our definition is the first to make such a clear formulation of the conditions, the method of checking conditions that is constantly used in colorimetric practice in fact conforms to this definition. It consists in presenting the same radiation (usually "white") in both fields. This requires that image sequencing be absent, and is the requirement that objectively identical radiations shall be visually equal.

(5) The conditions of comparison, while remaining colorimetric, may alter as to field size, nature of surround, preliminary adaptation, etc. Under each such condition it can be established which radiations are visually indistinguishable, so in principle one can say that under other different colorimetric conditions other radiations will be indistinguishable; i.e. colour equality can in principle be unstable and must depend on the conditions of comparison. We will not here discuss to what extent colour equality can alter under various colorimetric conditions of comparison, it being sufficient to note that the discrepancies are in any case not large.* The undoubted slightness of the deviations in colour matches under various colorimetric conditions of comparison is in principle of great importance. This alone enables us to use data derived from laboratory experiments for solving concrete problems, particularly those connected with the functions of the eye in a natural environment. This is an extremely general experimental result (quite apart from special experiments devoted to the purpose) and it shows that, at least within quite narrow limits, we may take colour matches as being stable.

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Since in any concrete problem we can neglect the variations in colour matching under varied colorimetric conditions, a more detailed specification of the conditions is evidently unnecessary. Detailed specification of the observational conditions may certainly improve the accuracy of observation. But it should not be assumed that the high accuracy would only relate to the conditions under which it was obtained. In particular such accuracy is meaningless in all applied problems, since the conditions under which the eye functions in a natural environment are extremely varied and differ no less from the conditions of observation in an apparatus than they do under various colorimetric conditions of comparison.

(6) No matter what the system of colour vision used, the problem of which radiations can be differentiated can be solved completely by comparing all possible radiations in pairs, i.e. by colorimetric experiments. The number of such comparisons is enormously large, and this method is extremely tedious. But Grassman's addition law is correct for colorimetric experiments, and this simplifies the problem enormously. The addition law is formulated thus: if any two radiations are visually indistinguishable, then after adding to both any radiation which is the same for both the fresh total radiations obtained will also be visually indistinguishable. Or in symbolic form, if $e_1(\lambda) \approx e_2(\lambda)$, then no matter what we take for $e_3(\lambda)$

$$e_3(\lambda)e_1(\lambda) + e_3(\lambda) \approx e_2(\lambda) + e_3(\lambda)$$

where the sign $\approx$ denotes that the corresponding radiations are visually indistinguishable.

The correctness of the addition law enables one to formulate the necessary and sufficient conditions for the radiations to be indistinguishable (and hence also distinguishable) in a fairly simple fashion, using the so-called addition curves.

If $a_1(\lambda)$, $a_2(\lambda)$, $a_3(\lambda)$, are linearly independent addition curves then a necessary

* Numerous papers deal with various colour matches under different conditions of comparison. While taking account of small variations of this type, it is very necessary to remark that, in the majority of papers, important assumptions are made which are not capable of proof, namely, that what has actually been found is what the authors assume they have found. The authors do not usually bear in mind either that the differing accuracies with which visual matches can be judged can readily be accompanied by other matches, or that the accuracy of data computed from the quantities observed directly may be much lower than that of the initial data.

and sufficient condition for two arbitrary radiations $e_1(\lambda)$, $e_2(\lambda)$ to be indistinguishable is equivalent to equality of the following integrals:*

$$\left.\begin{aligned} \int_0^\infty e_1(\lambda)a_1(\lambda)d\lambda &= \int_0^\infty e_2(\lambda)a_1(\lambda)d\lambda \\ \int_0^\infty e_1(\lambda)a_2(\lambda)d\lambda &= \int_0^\infty e_2(\lambda)a_2(\lambda)d\lambda \\ \int_0^\infty e_1(\lambda)a_3(\lambda)d\lambda &= \int_0^\infty e_2(\lambda)a_3(\lambda)d\lambda \end{aligned}\right\} \qquad (1)$$

(7) In order to determine the addition curves by experiment we first need to check the addition law, i.e. to check that for the vision system used there actually are functions which enable one to formulate the necessary and sufficient conditions for discrimination of the radiations in the form of equalities, as in (1). The addition curves, being functions which are completely determined, are meaningful only within those limits of observation and within the limits of accuracy for which the colour matches can be considered unchanged. In particular, when dealing with practical tasks it is senseless to use data which are of an accuracy considerably exceeding that corresponding to the limits of variation of colour matching under varied conditions of observation. The "average addition curves for man" need not be determined with an accuracy substantially exceeding that for individual variations in colour vision about the average of a large body of observers who may reasonably be considered normal.

Hence, in defining addition curves, the accuracy with which they have been determined at various points in the spectrum should always be stated, and with average curves for a number of observers the limits of scatter for the various individuals should be given.

If additivity is demonstrated for the given colour system, this implies that completely defined addition curves exist for it. Their experimental determination is most simply carried out as follows: using any trichrometric colorimeter the colour equations relating the colours of monochromatic radiations of different wavelengths to the three basic colours of the equipment are determined. The readings on the three scales will clearly be different at different wavelengths, and so may be considered as functions of wavelength: $\alpha_1(\lambda)$, $\alpha_2(\lambda)$ and $\alpha_3(\lambda)$.

In addition to the wavelengths, the energy fluxes of the monochromatic radiations must also be known (see above) where the fields are visually indistinguishable. These energy fluxes may be different at different wavelengths, varying with the characteristics of the equipment, i.e. they also are functions of wavelength, $\sigma(\lambda)$.

The functions $\alpha_1(\lambda)$, $\alpha_2(\lambda)$, $\alpha_3(\lambda)$, i.e. the readings on the equipment, are found by experiment. The function $\sigma(\lambda)$ must be determined as an inherent physical characteristic of the equipment. This is an objective characteristic, necessary to

* The three equations here correspond to the three linear addition curves for the foveal areas of normal trichromats. In principle these can be fewer (dichromats) or more (parafoveal areas of trichromats [2]). The discussion can readily be generalized to any case. The three equations are written only for the sake of the greatest possible clarity of exposition. We do not give the derivation of (1) from the addition law, merely referring the reader to the appropriate literature [5, 6].

ensure that the experiments are reproducible. Under these conditions the addition curves are determined from the measured data via the formulae:

$$a_1(\lambda) = \alpha_1(\lambda)/\sigma(\lambda);\ a_2(\lambda) = \alpha_2(\lambda)/\sigma(\lambda);\ a_3(\lambda) = \alpha_3(\lambda)/\sigma(\lambda). \qquad (2)$$

We still have to prove that the functions $a_1(\lambda)$, $a_2(\lambda)$, $a_3(\lambda)$, defined by (2) are actually addition curves, i.e. that they enable us to formulate the necessary and sufficient conditions for visual identity in the form of (1). This is not difficult to do if we start from the fact that any radiation may be considered as the sum of monochromatic radiations and apply Grassman's law. To save space, we do not propose to give this proof here, since it is fairly elementary and is almost a repetition of the deduction of (1).

In principle this discussion differentiates between addition curves as expressed by (2) and the "three colour coefficient curves" used by Guild, Wright and certain others. The "coefficient curves" are obtained by dividing the readings $\alpha_1(\lambda)$, $\alpha_2(\lambda)$, $\alpha_3(\lambda)$, by the sum of the readings. In this way the curve for $\sigma(\lambda)$ is eliminated, as it is not required in deriving the coefficient curves. This simplifies matters, but simultaneously deprives the data of any theoretical or practical significance. As we have pointed out above, to give only the relative spectral composition of the radiations (i.e. the wavelengths alone, and not the monochromatic energies) is insufficient for ensuring reproducibility of the experiment. The "coefficient curves" do not enable one to solve the problem of which radiations are visually identical, i.e. to answer the very important question which is uniquely dealt with by colorimetric experiments. While the addition curves enable one to solve a number of general theoretical and applied questions with the aid of (1), the coefficient curves by themselves are of no use, since even the experiments from which they are derived are only incompletely described by them. Using the coefficient curves one cannot even deduce at what readings visual equality would be observed even on the equipment from which they were derived, i.e. the experiments cannot be reproduced.

(8) The Conference of the International Illumination Commission in Zürich (1955) acknowledged that the standard based on the work of Guild and Wright was unsatisfactory, and we welcome this warmly. It is indeed to be regretted that the erroneous handling of the problem by these workers has only been acknowledged 25 years late, which could easily have been avoided. It is therefore very important to base any new work in this field on a more rigorous analysis of the problem and not on different arguments which are somewhat psychological and vaguely formulated.

As (2) shows, we need to know the readings of the equipment at different monochromatic wavelength settings in order to derive the addition curves, i.e. we need to know the spectral distribution for the equipment. In particular, there is no need to know in what system of basic colours the measurements were carried out. The basic colour system must, however, be the same in all experiments.

The misunderstandings of which we spoke above are widespread and pose the question as to what is implied by the "unchangingness of the three-colour coordinate system". All the radiations used experimentally must be determined in absolute spectral composition of luminous energy flux. This applies particularly to the basic colours used in the equipment. The basic colours are the colours seen in the equipment when the corresponding scales read 1, 0, 0; 0, 1, 0; and 0, 0, 1,

respectively. If the three-colour system is to remain unchanged the absolute spectral distributions of their fluxes must be unchanged at these readings. Otherwise the same objective settings on the equipment would be given a different numerical expression, which would denote a change in the co-ordinate system.

When the tricolour system of co-ordinates is not kept unchanged the numerical equality of the readings in terms of the various wavelengths of the type given above will not denote their being visually identical, i.e. the colour measurements will lose all meaning.

The lack of clarity in the concept "unchanged tricolour co-ordinate system" has occasioned many difficulties of another type in investigations. This will be made clear by an example. Wright [4] determined the addition curves for various observers and computed his readings in such a way that for all observers there was a certain radiation taken as "white", which was always specified by the numbers 1, 1, 1 (this was performed by all the readings for any given observer being taken on scales which were calibrated by the observer setting a mixture of the three basic colours to be equivalent to a "white" radiation).

In fact when comparing a "white" radiation with a continuous spectrum with a mixture of three monochromatic radiations, Wright's various observers made objectively different settings. Otherwise put, the equalities judged by some observers on equating with a "white" radiation were not agreed by others. These objectively different experimental results are given in identical form for all observers as 1, 1, 1 in Wright's report. Analogous results would certainly be obtained on using radiations other than white. Hence in those spectral regions where the various observers made the same matches Wright's numerical data are different, while for those regions where Wright's figures for different observers are the same the matches of some, and perhaps all, were different. The undesirability of Wright's mathematical operations is particularly clear when we consider his "mean" values for the various observers. If the observers all make the same match at some wavelength, then according to Wright they are all different. The computed "mean" will differ from the settings at least of some observers. What can this "mean" signify if all observers make the same match?

A particularly serious fault in Wright's calculation is that it is impossible to establish from the data he gives what the directly observed results were. Experiments carried out in different places would be incomparable, since agreement on numerical values does not denote agreement between the experiments, while numerical discrepancies do not denote discrepancies in the visual estimates. It scarcely remains to say that this is impermissible.

The analysis given here shows how much confusion has been introduced into colorimetry by the obstinate tendency to separate "brightness" from "colour" in colorimetry. This tendency, which has no basis in experiment, together with the neglect to define terms rigorously and argue logically, is the source of most of the errors and misunderstandings.

We must emphasize firmly that colour is multiform, or, more accurately, that the stimuli are multiform, being three-dimensional (only any four colours, of which three are independent, can be related to one another experimentally). So we must emphasize that objective colorimetric measurements must always be specified by three parameters,

and the radiations by their absolute and not their relative spectral compositions. If this had not been overlooked so frequently our discussion would not have been required.

CONCLUSIONS

The capacity to detect objective differences between radiations is for the visual analysor the sole source of correct information about the environment. A decision as to which radiations are visually identical is decisively and completely given by colorimetric experiments in which the fields are rendered indistinguishable, and by these alone. If colorimetric experiments are to be reproducible, the absolute spectral compositions of the radiations in the fields compared must be specified, and not merely the relative ones. The article gives a formulation of the "colorimetric conditions of comparison" which are both necessary and sufficient conditions that the colour is a function of the objective physical properties of the radiations compared.

Translated by J. E. S. Bradley

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A NEW METHOD OF STUDYING THE ACTIVITY OF VARIOUS PARTS OF THE RETINA

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(*Received* 10 *July* 1956)

For studying certain functions of individual retinal areas a technique has been developed in which by means of a suction cap with a special screen, shown in the diagram, any part of the retina can be excluded, and at the same time, if necessary, a recording of the eye movements during the perception process can be taken.

The outside diameter of the cap was 13 mm. The body of the cap 2 and the empty side-arm 9 attached to the cap were made of plastic. The empty side-arm was joined to the cavity of the cap by the channel 10. When the cap has to be very light, it is advantageous to make the body of thin (0·1 mm) duralumin with a wider (0·5 mm) and lightly goffered edge. The goffered edge attaches the cap to the eyeball, 1. Since the cap was without inertia the retinal image of the screen 6 could be kept quite stationary.

A circular cover glass 3 was fixed to the body of the cap, and to this a thin duralumin plate 8 with a diaphragm 7 is attached. A small mirror, 4, was fixed to the plate 8, as well as four legs, 5, made of very thin steel wire. The shield 6, made of paper foil, colour filter, or opal glass, was attached to the legs.

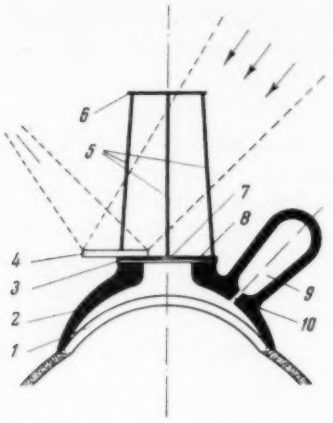

FIG. 1. Suction cap for the exclusion, or "masking", of various parts of the retina.

The mirror is used to record the light beam from the eye movements. The observer saw the objects of perception (the background) through the diaphragm 7 with one unshielded part of the retina, while with another part he saw the shield, this remaining fixed relative to the retina. By changing the diaphragm size, and the eye-shield distance, the required sharpness of the image of the shield was attained. By changing the shape and size of the shield it was possible to cover various parts of the retina during the perception process.

The surface of the opal glass shield of thickness 0·1–0·2 mm can be illuminated by light of given spectral composition.

The correct use of the cap is described in the article on the perception of a stationary retinal image [1].

Preliminary experiments showed that with constant illumination of the shield the region of the retina on to which the shield was projected remained as a blind spot for 2–3 sec after placing the cap in position, no matter what the brightness or colour of the illuminated shield, or the relations of these quantities for shield and background.

The results of a few experiments will serve to illustrate this statement. Circular shields were used in the experiments, these being made from opal glass or black paper, subtending an angle of 15° at the observer's eye, and covering the central part of the retina. The other eye was covered by a black eye-shield.

(1) When the opal glass was illuminated by a filament lamp to a brightness of 5000 lux it vanished and blended with the black background for 2–3 sec, after attaching the cap to the eye.

(2) A shield made of black paper was visible against a white background to the observer, and blended with it after 2–3 sec. The background was brightly illuminated white paper with lines drawn on it. The lines were so arranged that one of them should always fall within the field of vision of the subject during the experiment.

When the background (e.g. a sheet of Whatman paper) was uniformly illuminated, with the edges outside the subject's field of view, there being no detail on the background which could be presented to the retina on moving the eye, thanks to diffraction at the diaphragm, the nature of the background could not be perceived until after

the shield had blended with the background, when the background itself could be seen. The subject could make no statement about the colour or brightness of the background. It was sufficient to bring some object or other into the field of view to enable the observer to perceive the background at once, and a light touch on the shield, i.e. a slight movement of its image on the retina, was sufficient to cause the shield to reappear and be perceived at once.

(3) When the laboratory and its equipment formed the background, the shield, without regard to its colour, was perceived within 2–3 sec of putting on the cap as a dark grey or black spot, of colour approximating to that of the darkest area in the background. No deviations from normal were found in the perception of surrounding objects.

(4) In one of the experiments the background was an arrangement of sheets of paper, one of which was painted a saturated red colour, while another was painted a saturated blue colour. Two to three minutes after putting on the cap the observer looking at the red sheet, for instance, ceased to perceive the shield and saw a uniform red background. If the observer now transferred his gaze to the blue sheet at once an image of the shield appeared on the blue background, this being of a red colour less saturated than that of the red sheet, and lasting for 3–4 sec, after which it blended with the blue background. An analogous result was found on transferring the gaze from the blue to the red sheet, but the shield image had a blue hue in this case.

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CONCLUSIONS

A new technique in which any part of the retina can be excluded during the process of perception is described. The method can be used in a number of ways, primarily for studying the functions and interactions of various retinal areas during the process of perception.

Translated by J. E. S. BRADLEY

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THE VISCOUS-ELASTIC PROPERTIES OF DIFFERENT TYPES OF MUSCLES

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(*Received* 14 *September* 1956)

THE question of the ability of a muscular structure to respond to a stimulus by rapid or slow contraction has been discussed for many years from various viewpoints. Some investigators consider that the basis of these two types of muscular activity lies in the greater or lesser degree of functional mobility of the muscle tissue. Most of them are inclined to believe that tonic contraction is the result of a lowered

lability [1, 2]. As far back as 1892, Vvedenskii had developed his concept of a change in lability, and of the factor of the "adjustment" of an organ to a given working condition. The concept that reorganization of the functional mobility of the tissue depends on a change in the nature of the nerve action, which predisposes the muscle to either a tonic function or to the function of rapid tetanic contractions, is in accord with this point of view [3–6].

With regard to the actual nature and internal mechanism of the two types of contraction, there exist two fundamental points of view. On the one hand, tonus is considered to be the tetanus of slow, single contractions; it is assumed that the nature of rapid and of slow contractions is the same, and involves merely a quantitative difference in the structure of tonic (slow) and tetanic (rapid) fibres [7–9]. On the other hand, some authors consider that at the basis of the new type of contraction—tetanus—arising in the evolution of muscular tissue, there lies a fundamentally different mechanism, and stipulate an actual change in the contractile substrate itself [10]. From this follows the conclusion that there is a qualitative difference between muscles capable of tonic or tetanic contraction, and that independent rapid and slow fibres exist [11, 12].

Biophysical investigations on the mechanical properties of muscles play an important part in the elucidation of this subject. Such methods allow the investigation both of the details of the structural changes at the time of the contraction itself (of one or the other type) and of the comparative mechanical properties of the substance of the various muscles in the resting state. We consider that here is the means of answering in some measure the question of whether or not the structure and characteristics of the muscle substance itself play an important role in the formation of one or other type of contraction. In a whole series of experiments on the mechanical properties of muscles, carried out in the schools of Hill and Bukhtal and playing an important part in the development of the modern concepts of the biophysics of muscular contraction, no special effort was made to study the different types of muscles. However, some experiments on the investigation and comparison of the mechanical properties of functionally different muscles have already been recorded in the works of a number of authors [8, 9, 13, 14].

They showed that Hill's suggestion of a visco-elastic pattern for a muscle applied to various muscular structures, both striated and smooth.

A comparison of the mechanical properties of a number of striated and smooth muscles, carried out by Levin and Wyman [13], led to the conclusion that, "whatever the structure of these muscles, from the mechanical point of view they may be represented by a single visco-elastic model", in which besides the free elasticity there exists also a damping elasticity. All the types of muscles investigated, as these authors showed, gave similar exponential curves of length in relation to tension, and a typical sigmoid curve of work in relation to speed. Ritchie [8] also arrived at the conclusion that such different muscles as the masticatory muscles of the shark (rapid, striated) and the longitudinal muscles of the holothurian (slow, smooth) give quantitatively similar curves on tension and release, corresponding with the concept of the complex visco-elastic models of muscles. Ritchie suggests that "these muscles differ widely in speed, but are probably fairly similar in structure". Pryor [9] also found no qualitative differences in mechanical properties, and hence in molecular structure, of

smooth and striated muscle, and considered at that time that muscles with the function of tonic contraction have apparently stronger and more numerous interchain links (intermolecular side chains) than fast muscles. The basis of this view was that tonic muscles did not display a temporary fall in tension on rapid extension.

But in the above-mentioned works, the comparison of the mechanical properties of the various types of muscles was carried out without bringing in enough quantitative data, and often without evaluation of the separate mechanical parameters (viscosity and elasticity). In this respect, the work of Zhukov on the investigation of the mechanical properties of striated muscles of the frog [14] and the muscles of fresh-water molluscs [6] has the advantage over the previous investigations. This author showed that all these types of muscles, when subjected to load, show, as would be expected, a resistance to deformation which is connected both with their elasticity and with their viscosity. Change in length on extension takes place in two phases: first, a rapid increase in length at the moment of application of the load, and second, a slow subsequent extension. In Zhukov's opinion the first phase is the expression of the elastic properties of the muscle, and the second phase is determined by the viscous "pliability" to stretching, or plasticity. Plastic deformation has its limit. Having observed both the stages of deformation—on the one hand, in the white and yellow parts of the adductor of the fresh-water mussel, and on the other hand in the striated muscles of a frog—Zhukov expresses the opinion that they differ only quantitatively in their mechanical properties. This quantitative difference is along two lines: firstly, the period of a single contraction of the adductor (closing) muscle of the mollusc is many times greater than the period of contraction of the sartorius muscle of the frog, and secondly, viscous properties are exhibited more clearly by the tonic muscle; "the viscous pliability of the adductor muscle to stretching is so great that it behaves like a plastic body whilst the viscous pliability of striated muscles is considerably less".

However, both the treatment of the data obtained by Zhukov and the terminology he used for denoting the mechanical properties (for example, plasticity, viscous pliability, etc.) are liable to some objections, to which we shall refer below.

A certain amount of confusion in the definition of mechanical parameters in physiological literature, and the absence in the previously mentioned works of an analysis of the deformation curve by phases, gives us no means of investigating more deeply the qualitative peculiarities of each type of muscle.

The published data mentioned above is evidence that the question of the mechanical properties of various muscle structures is still not sufficiently explained and demands careful investigation.

When considering the mechanical properties of muscles it is extraordinarily fruitful and helpful to compare them with the visco-elastic properties of high-polymer compounds. The study of the structural and mechanical properties of the high-polymers has given origin to a series of new methods, allowing the analysis of the experimental results, and the judging of the peculiarities of the structure investigated.

METHODS

In the laboratory of Academician Rebinder [15, 16] a series of experiments has been worked out for the study of the mechanical properties of high-polymers by

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means of recording a family of curves of deformation with time at various constant loads. This method permits the calculation of a number of mechanical parameters and at the same time a quantitative estimation of the many aspects of the mechanical properties of the system under investigation. By means of this technique the elastic, super-elastic and viscous properties of the high-polymers were displayed, and are estimated in terms of the respective constants: (a) conditioned-"instantaneous" modulus of resilience; (b) the modulus of elasticity; (c) relaxation (true) viscosity, etc. The conditioned-"instantaneous" modulus of resilience really represents the elastic deformation developing at the speed of sound on applying a load. The modulus of elasticity corresponds to the full development of elastic deformation, whereby the super-elastic properties of the longitudinal molecular chains are particularly displayed. We have attempted to apply the method of deformation with time at constant load for the comparative study of mechanical properties of muscles differing in structure and function in terms of their seprarate mechanical parameters.

The following muscles were investigated: the sartorius muscle of the frog—a typical tetanic muscle; a transverse strip of stomach tissue—smooth, tonic muscle; the rectus abdominis muscle of the frog—a tonic striated muscle, capable of giving both rapid and slow contractions, and the adductor muscle of the fresh-water mussel *Anodonta*, giving prolonged tonic contraction. In all the comparative experiments equal strips of the muscle under investigation were used (length of the order of 8–10 mm, width 4–5 mm, thickness 0·5–1 mm). With the aim of studying the deformation of muscles under constant load an instrument was used which was constructed on the principle of a balance; on one arm of the beam hung a pan upon which the load was placed, and on the other a special clamp for holding the muscle. The weight of the pan was balanced by the weight of the clamp and of the strip of muscle. When equilibrium had been established, the other end of the strip of muscle was fixed in an immovable clamp. The construction of the instrument permitted the placing of various initial loads of 0·5–20 g on the muscle. The strip of muscle was mounted in a double-walled water-bath to keep it at constant temperature. For recording the deformation curves we used a photokymograph, on the aperture of which was projected a ray of light from a mirror mounted on the axis of rotation of the beam.

EXPERIMENTAL RESULTS AND DISCUSSION

Under the action of a constant load and subsequent unloading two phases of deformation are observed: a rapid extension (of the order of 1 sec) and a subsequent slow increase in length. Unloading gives the opposite picture: first a very rapid shortening, then a slow return to the original length, which always remains incomplete due to residual deformation. The deformation curves on loading muscles and the high-polymers show a great deal of similarity, as a result of which we tried to evaluate the mechanical properties of muscles with parameters worked out for high-polymers. However, we shall call the first phase the phase of conditioned-"instantaneous" elastic (and not resilient) deformation, since under the conditions of this experiment we had no chance of estimating the strictly resilient deformation. The second phase we have denoted as the phase of actual elastic deformation. The "instantaneous" modulus of elasticity corresponds to the "instantaneous" deformation, and the modulus of temporal-elastic deformation to the temporal deformation.

In Fig. 1A is shown a deformation curve of the sartorius muscle of the frog having an almost π-shaped form. The basic stretching of the muscle occurs in the first phase, whilst in the second phase the increase in length is slight. The angle of incidence α with the straight part of the flow curve is very small. Removal of the load causes rapid shortening, equal to the stretching in the first phase, and a residual deformation is observed which forms 10–20 per cent of the total deformation with the load of 5 g and an application of the load for 50 sec. A curve of this nature can be considered typical for tetanic muscles.

The extension curve for the frog rectus abdominis muscle (Fig. 1B) shows a distinct difference from the extension curve of the tetanic muscle. In comparison with the sartorius, this muscle, under the action of the same load, shows a considerably greater total stretching with regard to both the increase in the "instantaneous" deformation and the gradual lengthening in the second phase. However, the phases do not both increase proportionally, since the temporal deformation in the

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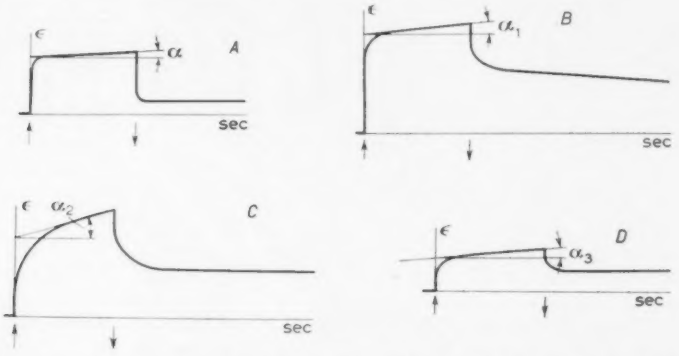

FIG. 1. Deformation curves of various types of muscles under the action of a constant load. Load, 5 g: duration of load 50 sec: arrows mark the times of application and removal of the load. ε = degree of deformation; (A) sartorius muscle; (B) rectus abdominis muscle; (C) stomach muscle; (D) adductor muscle (white part) of *Anodonta*.

rectus muscle forms a considerable part of the total deformation. The path of the curve in the second phase is more steep ($\alpha_1 > \alpha$). After the "instantaneous" extension a smooth "flowing-out" is observed, which is poorly expressed in the case of the sartorius muscle. The reverse process of shortening after unloading also takes a very different course. After a rapid shortening, which is usually less than the stretching after loading in the first phase, return to the original length takes place very slowly and a large amount of residual deformation is left (of the order of 50 per cent of the total deformation) under the same conditions as mentioned above for the sartorius muscle.

The extension curve for the transverse strip of stomach tissue is characteristic (Fig. 1C). The first phase is not expressed so strongly as in the rectus muscle. In this case, the whole deformation is confined for the most part to the second phase. A considerable "flow", i.e. plasticity, is visible. Unloading reveals a high residual deformation, of the order of 50–60 per cent of the full deformation, which is practically irreversible.

Finally, in Fig. 1D is shown a curve of the development of deformation under the action of a constant load on a strip of the white part of the adductor of *Anodonta*. The first thing that strikes the eye is that, other things being equal, the muscle of this mollusc stretches far less than all the enumerated frog muscles. In this case, the well-expressed first phase, determining the full deformation, and the gradual slight increase in length during the time the load is acting are characteristic. After unloading, a large residual deformation remains (about 40 per cent). This low capability of extension is evidently somehow connected with the adducting function of the muscle.

In Table 1 are some quantitative data giving evidence of the various mechanical properties of the types of muscles studied.

TABLE 1

Types of muscles studied	Cond.-"instant" deformn. ε_1	Modulus of cond.-"instant" deform. E_1	Cond.-"instant" modulus on unloading E_1	Temporal elastic deformn. ε_2	Modulus of temporal elastic deformn. E_2	Total deform. ε_3	Residual deform. in % of total deformn.	Ratio of moduli E_1/E_2	Angle α (°)
Sartorius	16	0·31	0·33	2·5	2	20	19	0·155	3
Rectus abdominis	25	0·2	0·63	9	0·55	36	44	0·36	7
Stomach	13	0·38	0·46	15	0·33	41	50	1·15	17
Adductor of *Anodonta* (white part)	7	0·71	1·25	4	1·25	12	40	0·56	5

The two-phase increase in length of the various muscles under the action of a constant load is a reflection of their elastic properties, as follows from analogy with the high-polymers. It is not in agreement, for example, with Zhukov's treatment of the results he obtained. According to Zhukov, only the first phase is elastic, whilst the second phase is explained by viscous pliability or plasticity. Zhukov characterizes each muscle substrate by the appearance of elastic and plastic properties, the latter of which he identifies with viscosity. It seems to us that an inaccuracy in definition has been introduced here; by plasticity is implied mainly an irreversible change in structure, and not an internal friction or viscosity. Together with this, the statement by Zhukov that the second phase of the deformation curve only expresses plastic deformation seems inaccurate, since the author himself evidently has in mind the viscous change of the substance (the viscous "pliability"). It would appear that the deformation in both the first and second phases is by nature the same thing, but its development in the second phase is affected by the viscosity.

If we follow up the suggestion that the molecular chains of the muscle proteins are in some way folded or wrapped round each other, then under the action of the applied load there will take place firstly a change in the chain itself (a change in the inter-atomic distances and valency angles) and secondly a straightening out and unwrapping of the chains. However, according to the degree of stretching, the straightening out will be made more and more difficult by the elastic tension developing along the chains, and also by the increasing resistance of the viscous elements and

inter-chain links. In this way it is possible to explain the change in the character of the elastic deformation in the second phase. After the prolonged action of the load in the case of tetanic muscles the stretching proceeds to a definite limit, and the deformation curve in the second phase approaches a certain definite level asymptotically. For mixed and tonic muscles it is characteristic that in the process of stretching, within the practical limits of the experiment, a definite upper limit of deformation is not reached, and the curve in the second phase changes to an inclined straight line. This is shown well by smooth muscle (stomach muscle). This is to be explained by the idea that the application of a load at a definite degree of extension may prove critical and cause irreversible structural changes (for example, of the intermolecular bridges). The degree of this disturbance in the structure may be judged from the residual deformation, which is also partly linked with the damping action of viscosity. The influence of the latter is shown by the slowness of the shortening of the muscle after unloading. However, a complete return to the original length is not observed, and after unloading after a definite interval, the curve becomes parallel to the abscissa. The residual deformation in this part is evidently closely linked with plastic (irreversible) deformation, i.e. with changes in the structural network, and not with viscosity.

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Comparison of the results obtained shows that the maximum deformation (greatest extension) is seen in the stomach muscle, and this is determined basically by the second phase, which goes over into the characteristic "flow" of the muscle under tension connected with its plasticity. Least plastic is the tetanic sartorius muscle, in which full deformation is achieved almost "instantly", i.e. in the first phase, and the subsequent lengthening in the second phase is negligible.

In the whole series of experiments the interesting fact was noted that the "instantaneous" modulus of deformation increased on unloading (in the frog rectus, stomach muscle and mollusc adductor). This phenomenon, by analogy with the high-polymers, may be termed the "stabilizing" of the structures on deformation. The increase of the modulus, equivalent to the decrease of deformation, probably proceeds by way of the change of part of the elastic deformation to plastic deformation, with resulting irreversible disturbances in the structure: in this new state the structural elements possess different elastic properties, which are demonstrated by the increased "instantaneous" deformation modulus on unloading. On the other hand, the magnitude of the residual deformation is also an index of the plasticity of the tissue. It is interesting to note that it is just in those cases of a considerably decreased "instantaneous" deformation on unloading that the residual deformation is usually greater (as in the stomach muscle, for example), which is evidence of the high plasticity of these types of muscles. In the frog sartorius muscle, the function of which is rapid phasic contraction, a similar increase in plasticity (irreversible structural change) at the expense of its elastic properties is practically absent. The comparatively negligible residual deformation of this muscle is also evidence of its low plasticity.

Together with all these comments on the parameters mentioned in the comparison of the deformation curves of the various types of muscle, the value of the ratio between the temporal and conditioned-"instantaneous" deformation (E_{inst}/E_{temp}) is also to some extent characteristic. By this relationship we were able to judge the role of viscosity in the process of deformation and the degree of internal friction in the deformation of muscular structure.

From Table 1 it follows that this ratio has its least value in the case of the sartorius muscle and most in the case of the stomach muscle.

A comparison of certain parameters of the mechanical properties of the white and yellow parts of the adductor muscle of *Anodonta* (Table 2) makes it possible to correlate the mechanical and functional natures of these muscles (Fig. 2). Under

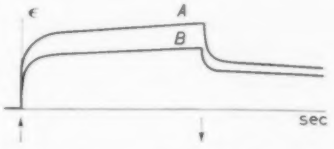

FIG. 2. Comparison of the deformation curves of the white and yellow parts of the adductor muscle of *Anodonta* under equal loads. Load, 5 g: duration of load 90 sec: arrows mark times of application and removal of the load. (A) the yellow part of the adductor; (B) the white part of the adductor.

identical experimental conditions under the influence of the same load the stretching effect was greater in the case of the yellow muscle, which functions basically as a rapid closer of the shell. The white muscle, by the activity of which the adducting tonus is maintained, is considerably less extensible. A comparison of the moduli of conditioned-"instantaneous" deformation shows that the elastic resistance is greater in the white muscle. The temporal deformation elasticity is greater for the yellow muscle. Evidently the white muscle has greater viscous resistance, which can also be deduced to some extent from the degree of residual deformation after unloading. The white muscle is distinguished by its greater plasticity, which allows it to "freeze" in prolonged tonus. However, in some respects both parts of the adductor muscle show considerable similarity. Thus, for example, the above-mentioned effect of the "stabilizing" of the structure is of the same order in both the white and the yellow muscles. The ratios of "instantaneous" and temporal deformation are also approximately equal. So apparently one cannot establish an absolute contrast between the two mechanical properties of parts of the adductor just as one cannot separate them even functionally, since it is known [17] that the white muscle is capable of giving weak phasic contractions and the yellow can develop a prolonged, admittedly weak, tonic tension.

TABLE 2

Type of muscle studied	Cond.-"instant" deformn. ε_1	Modulus of cond.-"instant" deformn. E_1	Cond.-"instant" modulus of deform. on unloading E_1	Temporal elastic deformn. ε_2	Modulus of temporal elastic deformn. E_2	Total deformn. ε_3	Residual deformn. as % of total deformn.	Ratio of moduli E_1/E_2
Adductor of *Anodonta* (white part)	10	1	2	12·5	0·8	24	60	1·25
Adductor of *Anodonta* (yellow part)	14	0·715	1·4	16	0·63	33	45	1·13

Thus, by means of a comparison of mechanical properties as shown by deformation curves at constant load of the types of muscles studied, we succeeded in discovering distinct differences between them. For each type of muscle the most characteristic values determining the nature of the deformation curve are: (1) the ratio of the temporal and the conditioned-"instantaneous" deformations; (2) the angle of incidence α of the straight line part of the flow curve, which points to the instability of the structure; (3) the magnitude of the residual deformation, as an index of the irreversible changes in the structure. These differences concern primarily the structures of the muscles investigated, which, we suggest, play no small role in the nature of the contraction.

An analysis of the deformation curves shows that the differences between the types of muscles described are not only of a quantitative, but also of a fundamental character, indicating peculiarities in the structural organization of the muscle substance.

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CONCLUSIONS

(1) With the aid of a method of determining the curves of deformation with time at a constant load a quantitative analysis and comparison of the mechanical parameters of various types of muscles was carried out.

(2) The elastic deformation in all the types of muscles investigated appears as a two-phase process, though the development of deformation by phases differs essentially in the various muscles.

(3) Characteristic of tetanic muscle is an almost instantaneous development of complete elastic deformation in the first phase, which points to the high elastic properties of these muscles and the small slowing effect of viscosity. In mixed and smooth muscles the development of elastic deformation continues into the second phase, where the factor of viscosity is more apparent, and this can be judged by the relationship between the moduli of "instantaneous" and temporal deformation.

(4) Elastic deformation is accompanied by irreversible changes in the muscle structure. An indication of the instability of the structure is given by the angle of incidence of the straight-line section of the curve of flow and by the amount of residual deformation. Of the types of muscles studied, tetanic muscles are least plastic, and their structure is easily resumed after the load is removed; this obviously corresponds with their characteristically phasic type of contraction.

I wish to thank Academician P. A. Rebinder for his interest in this work and for his valuable advice.

Translated by J. J. OLIVER

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THE DEVELOPMENT OF RADIATION AFTER-EFFECTS

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(*Received* 15 *November* 1956)

IONIZING radiations cause various physico-chemical, biochemical and functional changes in the living organism, these changes having an undulating character. In the main, two waves of such disturbances are observed, distinguished, more or less, by the period of time during which no symptoms are observed [1, 2].

In Patt and Brues' opinion [3], "during the latent period many decisive events must occur, these presumably being of a chemical nature". Dale [4] referring to the similarity of the biological action of ionizing radiations and radiomimetic substances, remarks that at the present time the hypothesis that chemical reactions develop from the moment of absorption of the radiation energy up to the final biological effect rests on a firmer experimental basis than previously.

From physico-chemical kinetic laws Tarusov [5] has proposed that the two waves of disturbance are caused by the development of two different, independent groups of reactions. In this connection it was of interest to try to study from general indications the mode of development of the changes arising in radiation sickness starting from the moment of irradiation to its final phase.

MATERIALS AND METHODS

As object of our quantitative study of radiation after-effects we used a clone of *Pelmatohydra oligactis*, isolated from the hydra population of the large pond in the new territory of the Moscow Zoo. Mature non-budding individuals of mean length 10 mm were irradiated. After irradiation, the 10 specimens were placed in beakers with 50 cm^3 of water, which was changed each day. The food supplied was *Daphnia*, given every other day.

The irradiation was carried out with an X-ray tube used for structural analysis (30 kV, 12 mA, 10 cm, distance, dose-rate 300 r/min). The 10 individuals were irradiated in test-tubes half filled with water, these being of height 20 mm and diameter 5 mm. In cases where measurements were carried out during the irradiation they were placed in Pasteur pipettes filled with water, one specimen in each.

In this work we took account only of the duration of the latent period, and not of the dynamics of the obvious pathological disturbances, since the duration of the latent period can itself serve as an index of the rate of development of the reactions leading to the various biochemical and physiological changes.

The change in the geometrical dimensions of the body was used as a criterion, this being a characteristic response of these organisms to ionizing radiation [6, 7, 8]. As our first general index for all stages of damage we used the ratio of the mean maximum length of the body, l_x, in the irradiated hydra to the same magnitude in the controls, l_n.

The amplitude of the periodic spontaneous movements was taken as a second index. In addition, we studied the peculiar contraction of the gastric cavity in the irradiated hydras, leading in the fed animals to the ejection of undigested food. The time when this reaction occurred could be clearly recorded by the ejection of the swallowed *Daphnia* from the oral aperture.

EXPERIMENTAL RESULTS

Fig. 1 shows the results obtained for the ratio l_x/l_n as a function of the time after irradiation at different doses. Curve A shows the change of this parameter for a dose of 35,000 r. The rapidly developing contraction is accompanied by early death: the whole body is observed to break up even only 3 hr after irradiation by this dose. Curve B relates to doses of 7000 r. Doses in the 5000–10,000 r range cause a fall in this ratio only after a day, on an average, the normal condition being restored during the following 2–3 days. The hydras recover completely and can again catch and consume *Daphnia*, but die, however, 20–45 days after irradiation. This dose range

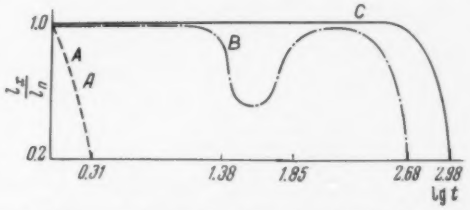

FIG. 1. Development of damage during the time after irradiation at the following doses: A, 35,000 r; B, 7,000 r; C, 2500 r.

causes the most marked undulatory nature of the disturbance. It should be mentioned that the control hydras lived for a year or more.

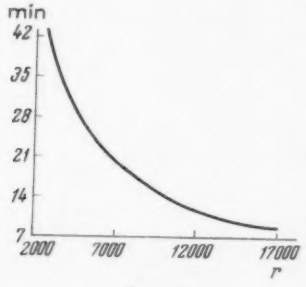

FIG. 2

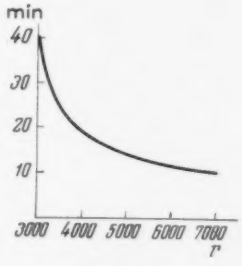

FIG. 3

FIG. 2. Relation between the latent period for the second wave and the dose.

FIG. 3. Relation between the latent period for the first wave of damage and the dose.

Doses of 2500–3000 r (curve C is for 2500 r) cause a change in l_x/l_n only after a latent period of several weeks. The first wave of disturbance is then not observed. When $l_x/l_n = 0{\cdot}20$, the hydra dies from complete reduction of the body; the stem breaks down as a whole.

The results for the relation between the duration of the latent period of the second wave of radiation reaction and the dose are shown in Fig. 2. Doses less than 2000 r cause no disturbance at all. As the dose is raised, the latent period falls exponentially. Fig. 3 shows the dose/latent period curve for the first wave. The digestive cavity is found to contract at doses over 3000 r, the time for the effect to appear then falling off exponentially with increasing dose.

In a further analysis of the nature of the early and remote changes in the irradiated organisms, the dependence of the latent period on temperature was investigated for both first and second waves (see Table 1).

TABLE 1. DEPENDENCE OF INCUBATION PERIOD ON TEMPERATURE

Temperature (°C)	Latent period			
	First wave		Second wave	
	Dose (r)	Mean duration (min.)	Dose (r)	Mean duration (days)
11	7000	$41 \pm 0{\cdot}43$	6000	$37 \pm 0{\cdot}62$
21	7000	$21 \pm 1{\cdot}61$	6000	$10 \pm 1{\cdot}36$

We took as the end-point of the latent period the point where a clearly visible shrinkage had begun ($1_x/1_n = 0{\cdot}7$). It was noticed that at low doses the longer the time of the incubation period before the reaction appeared, the more rapidly the hydras died from the disturbance produced.

The results of the thermal measurements are shown in Fig. 4; they give a temperature coefficient for the first wave of $Q_{10} = 2$, and for the second wave of $Q_{10} = 3{\cdot}5$. The temperature range used lies within that in which this type of hydra normally flourishes [9].

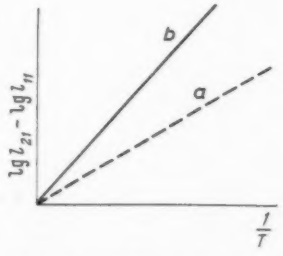

FIG. 4. Relation between the latent period and the temperature, plotted in Arrhenius co-ordinates: abscissa—reciprocal of the absolute temperature, T; ordinates—difference of the logarithms of the durations of the latent period at 21° and 11°. The straight lines (a) and (b) relate to the first and second waves respectively.

The rapid development and disappearance of the shock phenomena in the hydras (Fig. 1) may be explained as due to the direct action on the protoplast of the reaction products formed during the time of irradiation. As examples of such an action these will serve the initial activation of protoplasmic movement in irradiated amoebae [10], the stimulation of locomotion in mosquito larvae and chrysalises during irradiation [11] and the marked signs of excitation in rabbits, dogs, monkeys, and man [12]. But the changes during the first period of damage lead on to death only on forcing the dose, as is clear from Fig. 1 (curve A). This latter fact leads one to suppose that shock is the basic cause of radiation sickness [13], but this is evidently only true for high doses. Doses that do not cause a state of shock in the hydras lead to their later death, from the slowly-developing basic radiation damage (Fig. 1, curve C).

Our data on the dependence on temperature of the latent period for the second wave (Fig. 4) are confirmed in a number of investigations. Thus the temperature coefficient of the delay of the death of irradiated mature frogs, as calculated from the data in the literature [14], gives a value greater than 3.

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CONCLUSIONS

The results obtained may be explained by there being two reactions of different nature at the basis of the wave-like character of the radiation sickness in Hydra. The reactions of the first group, which cause shock or the early death of the hydras, have a normal temperature coefficient and a short latent period. Those of the second group, on the other hand, are initiated by much smaller doses, and have a higher temperature coefficient, together with a long latent period. These parameters, taken together, enable us to identify the processes responsible for the rapidly arising and quickly disappearing shock phenomena as being chemical straight-chain reactions. The second effect is clearly of autocatalytic type. It is very likely that the delayed radiation effects in Hydra are due to branched-chain reactions.

Translated by J. E. S. BRADLEY

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CERTAIN IRREGULARITIES IN THE GROWTH OF MACRO-COLONIES AFTER IRRADIATION OF YEAST CELLS WITH GAMMA RAYS FROM RADIO-COBALT

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(*Received* 27 *June* 1956)

THE first investigation of the action of ionizing radiations on micro-organisms was published as early as the end of last century [1]. The author of that work limited himself to a simple demonstration of the damaging effect of such radiations on certain protozoa. The next 20 years saw a rapid development in this field of radiobiology, and the accumulated experimental data allowed Blau [2] in Germany, and Crowther [3] in England, to arrive, almost simultaneously, at a generalization in which the theory of probability was applied to the radiobiological effect. It was these authors who proposed the well-known formula:

$$S = \frac{N}{N_0} = e^{-\alpha D}\left[1 + \frac{\alpha D}{1!} + \frac{(\alpha D)^2}{2!} + \ldots + \frac{(\alpha D)^{p-1}}{(p-1)!}\right]$$

where

N = number of cells surviving irradiation
N_0 = initial number of cells before irradiation
D = dose in relative units
α = probability of an effective quantum hit on the sensitive target volume of the cell
p = number of hits in the sensitive target volume required to inactivate the cell.

This formula, derived from Poisson's distribution law, is referred to as "Crowther's equation": it describes satisfactorily the relation between the dose and the resulting radiobiological effect. At the same time the so-called target theory was proposed and in the thirties was developed by several workers [4, 5]. This theory constitutes the first real attempt towards the interpretation of the statistical nature of the radiobiological effect. As transpired afterwards, the target theory could explain satisfactorily only a quite limited set of phenomena, viz. inactivation of viruses and of certain bacteria, and also the genetic effect. However, in an overwhelming majority of other cases a similar explanation was shown to be inapplicable [6]. Nevertheless, up to now no acceptable hypothesis has been proposed to replace the target theory in accounting for the statistical nature of the radiobiological effect that manifests itself particularly clearly in the case of irradiation of micro-organisms.

In the course of the thirties and forties, attention was focused, in the majority of studies, on the quantitative relation between the radiation dose and its final effect, e.g. inactivation; the processes leading to the damage were left out of account. The technique of investigation also developed in the direction of increased accuracy of

the determination of the end effect. In such studies, the most prevalent method seems to have been the counting of colonies visible to the naked eye, growing on an agar medium from irradiated micro-organisms [7].

In what, however, do the intermediate processes occurring between the preliminary radiochemical reactions and the end effect of inactivation of the irradiated micro-organisms consist?

According to the target theory, damage to the sensitive region leads to an immediate and irreversible suppression of cell division. However, data have accumulated in the literature that indicate that the problem is of far greater complexity.

As far as we know, the formation of non-typical di-cellular forms by irradiated yeast, *Saccharomyces cerevisiae*, was described for the first time in 1931 [8]. Within the next two years, it was announced that the delay in the division of *Euplotes taylori*, caused by sublethal doses of X-rays, does not occur immediately but some time after irradiation [9]. Later it was shown that exposure of *Dunaliella salina* [10], *Paramecium caudatum* [11, 12] and *Amoeba proteus* [13] to suitable doses of ionizing rays may cause death, several days or even weeks after the irradiation. Evidence both of delayed death and possibility of recovery of isolated individuals after fairly prolonged "radiation sickness" were confirmed using *Paramecium caudatum* after irradiation by ultra-violet [14], and in the cases of *Endamyces Magnusii* [15], *Colpoda* sp. [16] and other objects [17] after exposure to ionizing radiations. Such facts led Tarusov [18] to conclude that the existence of such a latent period after irradiation is a general characteristic.

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However, if we wish to unravel the mechanisms of action of penetrating radiations on micro-organisms, it is not sufficient to establish the existence of a latent period or the effect of a recovery in some or other individual cases. It is essential to investigate as thoroughly as possible the quantitative aspects of these phenomena, e.g. relationship between the percentage of reversibly inactivated cells and the dose; the kinetics of the development of the damaging processes, as well as the recovery of the irradiated micro-organisms. Lack of systematic studies of this nature has encouraged us to take some steps in that direction. The following work appears to be the first attempt to describe quantitatively the after-effects of radiation, observed on irradiated micro-organisms still capable of forming macro-colonies on a solid medium.

METHODS

The study was carried out using diploid yeast *Saccharomyces vini*, strain Megri-139-V, kindly given to us by V. I. Kudriavtsev. The selected strain better than others satisfied the requirements of a quantitative analysis in great regularity of cell size as well as in its inability to form clusters.

In each series of experiments we used our own laboratory strain developed from a single cell.

A suspension of resting yeast cells of uniform size was obtained by 48 hr incubation, at 30°, on a must-agar slope of strength 5° Bal. The size of the resulting cells was $8{\cdot}5 \pm 0{\cdot}33$ and the number of budding cells did not exceed 0·1–0·2 per cent. The preliminary runs disclosed that other durations of incubation and culture methods (e.g. in a liquid nutrient medium) produced inferior results.

The cells thus obtained were washed and suspended in sterile tap-water. This

suspension was diluted to give approximately 300,000 cells/ml, and distributed in 4 ml portions into glass containers, 17 mm in diameter. The irradiation was carried out with ^{60}Co using apparatus GUT-Co-400 (at half charge), the distance from the source of radiation being 5·5 cm. The mean energy of radiation source was 1·25 MeV and the dose intensity 820 r/min.*

At regular intervals, the irradiated suspension was carefully shaken and samples were withdrawn which, after suitable dilution, were sown on must-agar in a Petri dish. In the determination of the dimensions of the colonies, the control and experimental samples were diluted until the number of colonies developed in each Petri dish was approximately the same.

The number of cells capable of forming macro-colonies was determined by counting the colonies visible to the naked eye in the Petri dishes. In contrast to the standard method, the count was carried out four times: after incubation at 30°, for 48, 72, 96, and 120 hr. The dimensions of the colonies were determined using microscope MBI-1, fitted with a micrometer eye-piece, the measurements being carried out at magnification 2 x 8.

Blank samples taken from the experimental container before irradiation, as well as samples from the control vessel, were used.

EXPERIMENTAL RESULTS

Number of macro-colonies

The relative number of macro-colonies developing from the irradiated suspension of yeast cells after different periods of incubation on must-agar is given in Table 1.

TABLE 1. MEAN PERCENTAGE OF MACRO-COLONIES DEVELOPING ON MUST-AGAR FROM THE IRRADIATED YEAST SUSPENSION

Incubation (hr)	Irradiation dose ($r \times 10^3$)					
	0	12·3	24·6	36·9	49·2	73·8
48	100 ± 1·9	96·3 ± 2·3	69·8 ± 3·0	25·2 ± 1·3	6·2 ± 0·33	0·21 ± 0·03
72	100 ± 1·9	96·6 ± 2·1	79·0 ± 3·8	41·2 ± 2·9	14·2 ± 0·40	2·01 ± 0·35
96	100 ± 1·9	97·3 ± 1·9	80·2 ± 4·4	42·5 ± 3·7	15·6 ± 0·41	2·23 ± 0·39
120	100 ± 1·9	97·3 ± 1·9	80·2 ± 4·4	42·5 ± 3·7	15·6 ± 0·41	2·23 ± 0·39
$E = \frac{M_{48}}{M_{96}} 100$	100	99	87	59	39	9

The data given in the above table show that an incubation period exceeding 96 hr does not lead to a further increase in the number of macro-colonies. Evidently the specified period is amply sufficient for the cells to develop their ability for unlimited reproduction, in so far as this capacity was not irretrievably lost as a result of the irradiation. E indicates what percentages of cells form a macro-colony after a standard period of incubation for 48 hr.

* The dose intensity was calculated according to the formula $D = \frac{140\, N_0}{F^2} e^{-0.0025t}$ where N_0 = original activity in mc; F = distance in cm from source to object; t = time in weeks from determination of N_0 to moment of irradiation.

The survival curves determined after 48 and 96 hr of incubation are shown in Fig. 1. The dependence of E on the dose is represented graphically in Fig. 2.

Dimensions of the macro-colonies

In Table 2 are given the mean values of $M \pm m$ and the standard σ^2 of the diameters of the macro-colonies, formed in the course of 48 hr incubation from cells irradiated with different doses of γ-rays.

Fig. 3 shows the relation between the squares of the radii and of the dispersion of the diameters of the macro-colonies on the dose.

The square of the radius in a given case may be taken as an approximate index of the mean rate of growth of the colony. Indeed, the average rate of growth is proportional to the number of cells in the colony, and this latter value for sufficiently large colonies having a flat, lens-shape of radius r may be approximately taken as being proportional to πr^2.

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TABLE 2. MEAN VALUE OF $M \pm m$ AND STANDARD σ^2 OF THE DIMENSIONS OF MACRO-COLONIES

Dose ($r \times 10^3$)	0	12·3	24·6	36·9	4·29
$M \pm m$	44·5 ± 0·69	38·4 ± 1·23	29·0 ± 1·29	25·3 ± 1·79	19·1 ± 1·33
σ^2	38	128	168	173	171

The distribution function of the linear dimensions of the macro-colonies is shown in classes in Fig. 4 for both control and experimental groups.

Discussion of results

The pronounced S-shape of the yeast survival curve is very striking (Fig. 1). The p coefficient of Crowther's formula, characteristic of this curve, can be determined

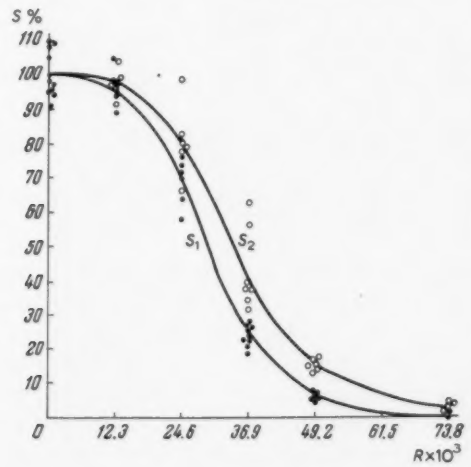

FIG. 1. Survival curves for yeast determined after 48 hr (S_1) and after 96 hr (S_2 of incubation.

by Ralston's method [10]. Its value is not less than 10 units.* Information available in the literature indicates that on irradiation of non-budding yeast cells, the value of p is different for haploid, diploid, triploid and tetraploid cultures [19], although the survival curve for budding yeast cells is invariably of an exponential form irrespective of ploidy [20]. However, the value of p obtained for resting diploid yeast cells in our experiments is higher than that reported by other workers. It is possible that the divergence in the slope of the survival curves is to be ascribed to variations in the wavelength. In fact, the data referred to relate to results obtained on irradiating yeast by either X-rays or by β- and α-particles of medium energies. On the other hand there is evidence that on the use of radiation of widely differing energy relations, the curve describing the damage by harder rays is distinguished by a greater degree of curvature, i.e. by a higher value of the coefficient p, than that relating to irradiation by comparatively softer rays.

Similar differences were observed on irradiating wheat seedlings and *Drosophila* eggs [21], yeast [8] and spores of *Rhizopus nigricans* [22]. In view of the hardness of rays used in the present work (1·25 MeV), we are justified in expecting analogous results in this case also.

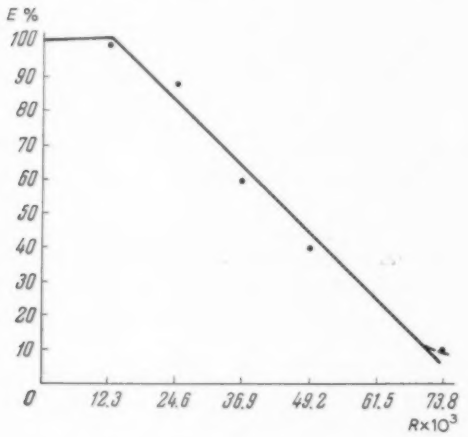

FIG. 2. Mean velocity of formation of macro-colonies by yeast irradiated with different doses of γ-rays.

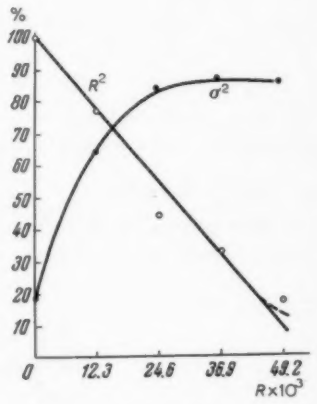

FIG. 3. Relationship between the dose and the square of the radius r^2 and the standard diameter σ^2, of the 48 hr macro-colonies.

However, we are interested not so much in the shape of the survival curves, as in the differences between curves obtained after 48 and 96 hr of incubation of the irradiated material (S_1 and S_2, Fig. 1). The difference, as is evident from Table 1, is determined by the growth of new macro-colonies after additional incubation for 24 and 48 hr. In other words, the rate of formation of the macro-colonies from the irradiated cells that have retained their ability for unlimited reproduction decreases markedly with the increase in the radiation dose, in consequence of which after a

* A more exact determination of the characteristic coefficients presents well-known difficulties, and was not carried out as a knowledge of such data was not essential for our purposes.

sufficiently high radiation dose, only a small porportion of such cells can form, in the first 48 hr incubation, colonies visible to the naked eye (in the controls, and also on irradiation with smaller doses, all the cells capable of unlimited reproduction, succeed in forming macro-colonies in this period of incubation). Hence, the value of the fraction of the cells may serve as an index of the relative rate of macro-colony formation, or of the relative mean rate of their reproduction. Let us denote this quantity by E. Then the relation between E and the radiation dose will be equal to the ratio of the two curves shown in Fig. 1,

$$E = f(D) = S_1/S_2$$

In the range $12{,}000 < D < 70{,}000$ r, E is directly proportional to D (Fig. 2), the equation expressing the relation between them being $E = a(1-kD)$.

The plateau in the range $0 < D < 12{,}000$ r is attributed to carrying out the count of the colonies over a considerable period of time (the first count after 48 hr of incubation, and then every 24 hr), and therefore, any possible difference in the rate of formation of the macro-colonies occurring up to the time of the first count cannot be revealed. To elucidate this question, we have studied the dimensions of these macro-colonies. The data similarly worked out for the dimensions of the 48 hr macro-colonies is shown in Table 2 and in Figs. 3 and 4.

In Fig. 3, the line denoted by r^2 illustrates the relationship between the mean square of the radius and the radiation dose. As was shown above, r^2 is proportional to the mean rate of reproduction of the cells forming a macro-colony, i.e. it represents the quantity E, but derived in a different manner. This statement is supported by the fact of the identical form of the relationship of S_1/S_2, or of r^2, to the irradiation dose.

Thus S_1/S_2, and r^2, are directly proportional to the dose, and satisfy the linear equation $E = a(1-kD)$. Further calculations show that the coefficient k is equal to 0·0161 in the first case, and 0·0183 in the second. This agreement is quite satisfactory, particularly if we take into account the comparatively rough method used in counting the macro-colonies.

Hence, we feel justified in concluding that irradiation by γ-rays causes retardation in the reproduction of the non-inactivated yeast cells, the mean rate of budding in such cells during the first 48 hr incubation at 30° being given by the equation $E = a(1-kD)$, for radiation doses of 0–70,000 r. In this equation, a represents the average growth velocity of the non-irradiated macro-colonies and the proportionality coefficient k indicates the extent of decrease of the mean rate of macro-colony growth after irradiation of the yeast cells with a unit dose (as a unit dose we have taken 1,000 r). In our case, $a = 100$ per cent and $k = 0{\cdot}0172 \pm 0{\cdot}0010$.

Let us now turn our attention to the graph of σ^2 in Fig. 3, which shows the relation between the degree of variance σ^2, of the linear dimensions of the macro-colonies and the radiation dose.* As is shown in Fig. 3, σ^2 at first increases rapidly with the increasing dose, and then having arrived at point $D = 24{\cdot}6 \times 10^3$ r, where its value is 4·5 times that of the control, σ^2 does not undergo any further change. Comparing Figs. 3 and 4, we observe that the plateau in the given case is attained at

* $\sigma^2 = \Sigma \dfrac{(m-M)^2}{n}$

a dose D, where the percentage of macro-colonies, formed after the supplementary incubation, has reached a considerable value. The rapid increase of σ^2 on passing from $D = 0$ to $D = 24 \times 10^3$ r indicates that the differences in the average dimensions of the macro-colonies seen in this range of doses are due to an irregular inhibition of budding of the various non-inactivated cells. In fact, if the retardation by the irradiation of the reproduction of the non-inactivated cells were uniform, σ^2 for the experimental groups would not differ from σ^2 of the control group of macro-colonies. We shall bring forward yet another confirmation for such a conclusion.

In the region from 0 to $12{\cdot}3 \times 10^3$ r, the value of σ^2 changes by 3·4 times, while the mean diameter of the macro-colonies changes by only 14 per cent and the number of non-inactivated cells by only 3 per cent. In view of this, and also since σ^2 is independent of the absolute dimensions of the macro-colonies, the magnitude of the variance of the diameter of the colony may be regarded as a suitable index of the radiation damage to the yeast cells, for quite low radiation doses, i.e. for doses that either nearly or completely fail to cause a lethal effect.

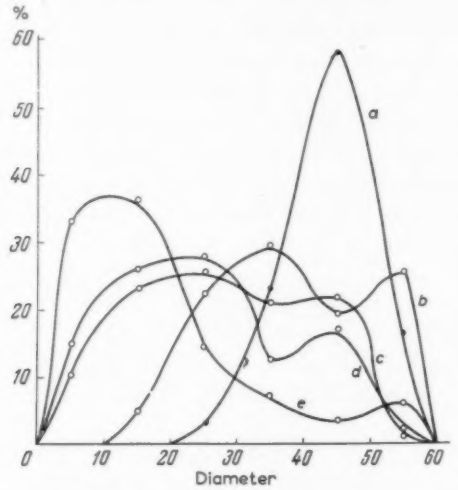

FIG. 4. Distribution of the diameters of the macro-colonies into groups for the control and experimental samples:

(a) control (c) $24{\cdot}6 \times 10^3$ r (e) $49{\cdot}2 \times 10^3$ r
(b) $12{\cdot}3 \times 10^3$ r (d) $36{\cdot}9 \times 10^3$ r

Finally, let us consider the graphs in Fig. 4. On separation into the groups 0–10, 11–20, 21–30, etc., of the aggregate diameters of the macro-colonies of the control cultures, we obtain a typical Gaussian curve with a maximum at 56 per cent in the range 40–50 (curve a). (Dimensions of the diameters are given in the scale units of the micrometer eye-piece at magnification 2×8.) As regards the distribution of diameters of irradiated macro-colonies, they are characterized by double-peaked curves. The range of doses at which the right-hand maxima of these curves occur nearly coincides with that for the maximum of the control curve. The ranges of doses for the left-hand maxima are progressively displaced further to the left the higher the dose for the corresponding aggregate of macro-colonies. Also, the right-hand side

maxima subside with increasing dose, whilst the left ones increase. Even for 12×10^3 r dose, inactivating only about 3 per cent of the cells, we can clearly observe the difference between the control and experimental groups in the variance of the linear dimensions of the macro-colonies (Fig. 3).

The double-peaked shape of the distribution curve for the aggregate diameters of the macro-colonies of the irradiated cells, and also the presence in each aggregate of colonies, not differing in their dimensions from the largest colonies of the control set, allows of the following conclusions. The individuals that after irradiation had not lost their power of unlimited reproduction apparently form at least two groups differing substantially in their mean rate of budding. One of these groups—the group decreasing with increasing dose—buds at the rate near to that for the non-irradiated yeast. The mean rate of budding of the remaining cells shows a considerable retardation, the degree of this retardation increasing with increasing dose of radiation, according to the above equation $E = a(1-kD)$.

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Thus although the considerable majority of the cells are not inactivated by radiation they are nevertheless damaged to a smaller or greater degree. It could even be supposed that all the irradiated cells are damaged to an extent differing for different cells—from insignificant inhibition to irreversible inactivation.

What is the nature of this damage to the cells that form a macro-colony more slowly than the control cells?

Such retardation of the growth of the macro-colonies may be explained either by reversible inactivation of varying duration, or by more or less constant inheritance of retardation of the tempo of budding; or finally, by the presence of both these effects.

All the above results show that the usual method of study of the radiobiological action on micro-organisms, by counting the macro-colonies, is not satisfactory for the deeper study of the mechanism of the biological action of radioactive radiations. Indeed, this technique permits the discernment of only the total final effect of the radiation damage, and gives no possibility of studying the different aspects of radiation damage and its development, or of recovery. There is no doubt, however, that the investigation of reversible and irreversible types of radiation damage in micro-organisms, the elucidation of the various forms of damage and their dependence on the dose are of significant interest in general radiobiology. Therefore, the development of more refined methods in the study of radiation after-effects on micro-organisms is an urgent and important task.

CONCLUSIONS

(1) By means of a determination of the macro-colonies after different time intervals and also by statistical analysis of the linear dimensions of 48 macro-colonies the presence of a radiation after-effect was detected in yeast cells irradiated with ^{60}Co γ-rays and retaining the property of unlimited multiplication.

(2) The radiation after-effect indicated is expressed in the decrease of the average rate of gemmation of these cells according to the rule $E = a(1-kD)$, where a is the average rate of gemmation of the yeast cells not irradiated. This relation of E to D appeared after 48 hr incubation and was observed for the whole range of doses investigated from 0 to 70,000 r.

(3) The irregularity of the degree of affection of the different uninactivated cells irradiated with the same dose is shown.

(4) A simple and sensitive method of registering the radiation after-effect in yeast organisms with low doses of irradiation by determining the degree of dispersion of the dimensions of the macro-colonies growing from an irradiated suspension on a solid medium is suggested.

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EFFECT OF X-RAYS ON THE PERMEABILITIES OF BLOOD-ORGAN BARRIERS

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(*Received* 20 *July* 1956)

THE presence of barriers between the generalized internal medium—the blood and extracellular fluid which bathes the tissues—and the transcellular fluid has been established over many years. The exchange between the blood and the medium in direct contact with the tissues is in the main controlled by selectively permeable barriers. The anatomical substrate of these barriers is the capillary walls, primarily the epithelia of these latter, which have specific properties in the various organs.

One barrier of particular importance is that between blood and brain, which provides a constant composition for the medium in direct contact with the central nervous system. The selective barrier properties are defined by the properties shown by the brain capillary endothelium, and also by the vascular brain plexus, the nerves and the ground-substance.

Many investigations concerned with cell-blood barriers have demonstrated their particular importance in removing foreign materials from the medium in direct contact with the organs, pathogenic agents in particular. It has also been shown that a number of physical and chemical agents affect the states of these barriers and that pathological processes must destroy them in order to develop and become localized.

Radiobiology and pathology, which have developed rapidly in the last decade, treat capillary permeability as one of the most important factors determining the biological action of ionizing radiation, and as playing a constructive part in the pathogenesis of radiation sickness. So far the effect of ionizing radiations on the blood-cell barriers has been inadequately studied. There are but few papers dealing with the effect of X-rays on the permeability of blood-cell barriers [1–9].

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The results obtained have been contradictory, evidently because of the varying experimental conditions (general or local irradiation, varying dose-rates, differing periods of study after irradiation, etc.) and of the various methods used to study the permeability.

New possibilities of studying permeability have been opened up by using radioisotope techniques. In this work we have studied the permeability of the blood-brain barrier, using radioactive phosphorus and iodine, at varying periods after X-irradiation. As controls we have used the permeability of the barriers in liver and muscle in all experiments.

METHODS

White rats were used in the experiments (mainly males), of weight 140–160 gm They were irradiated (800 r) on an RUP-1 unit, under the following conditions: 180 kV, current 10 mA, distance 30 cm, dose rate 31·4 r/min, filters 0·5 mm Cu, 0·75 mm Al, duration 25·45 min.

Radio-phosphorus (^{32}P) and radio-iodine (^{131}I) were used to test the permeabilities. They were injected intravenously directly after irradiation. The ^{32}P was used as $Na_2H^{32}PO_4$, 4 μc, the carrier being not more than 0·07 mg. The volume of solution injected was 0·5 ml. The ^{131}I was injected as $Na^{131}I$, carrier-free, 5 μc. The animals were killed by decapitation at various times after injecting the isotopes. The blood taken at the moment of decapitation was placed on a filter paper disc of area 2·83 cm^2. The brain tissue, liver and muscle were mounted (50 mg each) on discs of area 2·83 cm^2 after careful homogenization of the organs. Before homogenization the brain was freed from soft brain membrane and vascular plexus. An aluminium counter type AS-2 was used with the radio-phosphorus, an end-window counter being used with the radio-iodine. The counts/mg of dry tissue was computed. The organ activities were expressed as percentages of the activities of blood taken simultaneously. The ratios were used as permeability indices.

When radio-phosphorus was used the relative specific activity of the organ was also taken as an index of permeability: in the first experiments this was expressed as the ratio of the specific activity of the total organ phosphorus to the specific activity

of the total blood phosphorus, while in the later experiments this was expressed as the ratio of the specific activity of the acid-soluble phosphorus fraction in the organ to the specific activity of the blood inorganic phosphorus. The specific activity was expressed as the $^{32}P/^{31}P$ ratio, i.e. the counting rate per mg phosphorus.

In all cases the phosphorus was precipitated as $MgNH_4PO_4$. To determine the total phosphorus of blood and organs these were digested with a nitric-sulphuric acid mixture. The inorganic phosphorus in the blood was determined by precipitating it from the trichloracetic acid extract with a magnesia mixture. The acid-soluble phosphorus in the organs was separated after heating the trichloracetic acid extract with the nitric-sulphuric acid mixture, while the acid-insoluble phosphorus in these organs was determined by digesting the precipitates after the extraction. The precipitates obtained with the magnesia mixture were separated by centrifuging, freed from the magnesia mixture by washing with water, dried, and dissolved in NHCl. The activity was determined on an aliquot of solution, dried down on a metal disc of 2·83 cm² area. Fiske and Subbarow's method was used to determine the phosphorus content of another aliquot. Colorimetry was by photoelectric colorimeter, type FEK. Phosphorus determinations were done in duplicate. When the ^{32}P content of the blood inorganic phosphorus was determined, a certain amount of Na_2HPO_4 was always added before the precipitation.

EXPERIMENTAL RESULTS

Blood activity at different times after irradiation

Since the organ activities were expressed as percentages of the activity in blood taken simultaneously, the activity of the blood at different times after injection had to be established. The experiments directed to this end were carried out as follows: after injecting the ^{32}P by cardiac puncture, blood was taken from the femoral vein at 1, 5, 15, 30, 45, 60 and 120 min. The blood activity at different times after injection was expressed as a percentage of that taken at 1 min, this being taken as 100.

The results are shown in Fig. 1.

Curve a shows that the blood activity had dropped by 47·1 per cent at 5 min, and

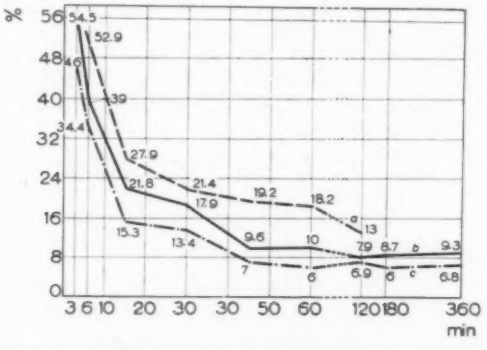

FIG. 1. Blood ^{32}P level at various times after injection into the heart. Abscissa—time in mins. Ordinate—per cent of ^{32}P in blood (1 min ≡ 100). Curves: a—blood taken repeatedly from the same animal; b—blood taken simultaneously from a group of control animals; c—blood taken simultaneously from a group of irradiated animals.

fell by 25 per cent in the next 10 min. After the first 30 min the activity fell 1–2 per cent per 15 min period. The blood activity as from 30 min after injection may be taken as having reached a plateau. Curves b and c were drawn from the mean results of many experiments carried out on different animals at various time intervals after the injection. Curve b is for the control experiments (69 in all), while c is for the irradiated animals(58).

All three curves run almost parallel, but in the experiments carried out with different animals both as controls (curve b) and as irradiated subjects (c) the plateau was reached somewhat later (at 45 min) and was at a lower level.

It is clear from these results that in the period when the blood activity is falling rapidly (i.e. shortly after the injection), the tissue activities, expressed as percentages of the activity in blood taken at the same time, are somewhat higher than the true ones. However, since this increase in the calculated values occurs both in the control and irradiated animals, it does not affect the accuracy.

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Blood-tissue barrier permeabilities at different times after irradiation.

In experiments where the animals were killed during the first 6 hr after irradiation the radioactive phosphorus was injected into the circulation immediately after the irradiation. When the animals were killed at 24, 48, and 72 hr after irradiation, the radiophosphorus was always injected 45 min before decapitation. The controls were killed at the same time after injecting the isotope as were the irradiated animals.

The results obtained are given in Table 1. The control experiment data give the distribution of the phosphorus injected into the circulation in the normals.

TABLE 1. EFFECT OF IRRADIATION ON THE DYNAMICS OF ^{32}P DISTRIBUTION IN ORGANS (DATA EXPRESSED AS PER CENT OF THE ACTIVITY IN BLOOD TAKEN SIMULTANEOUSLY. ^{32}P INJECTED IMMEDIATELY AFTER IRRADIATION)

Time (in min) after receiving 800 r	No. of animals		Brain		Muscle		Liver	
	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control
1	8	12	2·2	2·2	13·8	12·4	51	39·9
3	6	5	2·5	2·2	24·6	20·5	96·7	76·5
6	6	6	2·7	3·1	26	24	252·9	130
15	4	7	2·7	2·9	38	32	362	233
30	4	6	5·6	5·8	59	53	823	500
45	5	10	12·8	9·4	120	95·9	1440	746
60	5	4	14·3	8·6	211	99·4	1284	783·9
120	5	4	14·8	11·0	150	116·4	1118	749
180	5	5	25·9	15·7	201	176	740	595
360	5	5	20·0	13·4	173	161	768	774
24 hr*	5	5	6·5	9·4	122	95·9	1120	746
24 hr	3	—	20·0	9.4	106	95.9	937	746
48 hr	4	5	7·2	9·4	58·4	95·9	739	746
72 hr	9	5	4·4	9·4	18·5	95·9	345	746

* Radioactive phosphorus injected 45 min before decapitation.

^{32}P distribution in the organs of non-irradiated animals

It is clear from Table 1 that the greatest activity in the controls is found in the liver, and the least in the brain. Table 1 also shows that, while ^{32}P activity begins to appear in the liver and muscle even during the first minute after injection, ^{32}P only begins to appear in the brain at times between 15 and 30 min. The same tendency, with slightly different figures, is seen when the data are expressed as relative specific activities (Table 2 and Fig. 2).

Table 2. Effect of irradiation on the dynamics of ^{32}P distribution in organs (data expressed as relative specific activity)*

Time (in min) after receiving dose of 800 r	Brain		Liver	
	Irradiated	Control	Irradiated	Control
1	—	0·003	0·11	0·049
6	0·004	0·0039	0·32	0·19
15	—	0·026	—	0·71
45	0·053	0·037	3·8	2·04
180	0·091	0·065	3·06	2·2

* Specific activity of total tissue phosphorus divided by the specific activity of total blood phosphorus.

These data indicate that the radiophosphorus in the general circulation appears exceptionally slowly in the brain; they are in complete agreement with published data [10–18].

^{32}P distribution in the organs of irradiated animals

The permeabilities of blood-tissue barriers are altered by X-ray irradiation. Tables 1 and 2, and Fig. 2, show that the barrier permeabilities are raised even in the first few minutes after irradiation. This may be seen from the organ blood activity ratios, and from the specific activities. The permeability increase does not occur simultaneously in the various organs. The rise in the liver was observed at 1 min after the irradiation, while 3–15 min were required by muscle, and up to 45 min by the brain.

The maximum rise in activity was found at the following times: liver, 45 min; muscle 60 min, and brain 1–3 hr. The lower ^{32}P level in the blood during the first hours after irradiation (see Fig. 1, a, b, and c) agreed completely with the increase in barrier permeability.

Table 1 shows that at 48 hr after irradiation the blood-brain barrier permeability had dropped considerably, as had those of the barriers in liver and muscle. This drop was particularly marked at 72 hr after irradiation (this being the time when most of the irradiated animals died). The drop in the blood-brain barrier permeability was even observed at 24 hr after irradiation in some experiments. The high blood activity during the second and third days (Table 3) also indicates a permeability drop.

We now turn our attention to the fact that an increase in the absolute counting rate was found in fresh organs in the animals on the third day after irradiation, side

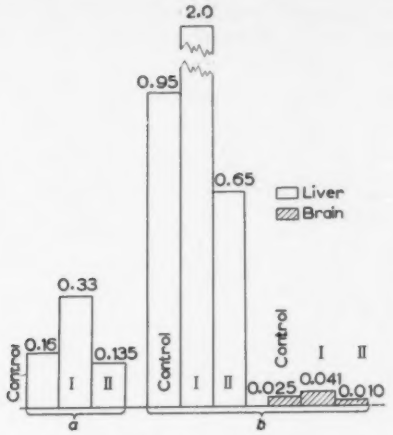

FIG. 2. Effect of X-rays on the ^{32}P distribution in organs. The data are expressed as relative specific activities (RSA); the specific activity of the tissue acid-soluble phosphorus is referred to the specific activity of blood inorganic phosphorus: I—^{32}P injected immediately after irradiation; II—^{32}P injected at 3 hr after irradiation. Animals decapitated after injecting ^{32}P: a—after 6 min; b—after 45 min.

by side with a sharp drop in permeability (i.e. a reduction in the organ/blood activity ratio and in the ratio of the specific activity of the acid-soluble organ fraction to the blood inorganic phosphorus specific activity). This apparently contradictory circumstance may be explained by the tendency of the organs to accumulate phosphorus rising at the same time as the barrier permeabilities fall.

TABLE 3. DISTRIBUTION OF RADIOACTIVE PHOSPHORUS IN THE ORGANS IN THE FIRST AND THIRD DAYS AFTER IRRADIATION (^{32}P INJECTED 45 MIN BEFORE DECAPITATION)

Tissue	Control		^{32}P injected immediately after irradiation		^{32}P injected 72 hr after irradiation	
	Counts/mg tissue	As per cent of activity of blood taken simultaneously	Counts/mg tissue	As per cent of activity of blood taken simultaneously	Counts/mg tissue	As per cent of activity of blood taken simultaneously
Blood	5·2	—	3·75	—	20	—
Brain	0·49	9·4	0·48	12·8	0·88	4·4
Liver	38·9	748	54	1440	69	345
Muscle	5	96	4·5	120	3·7	18·5

Some experiments were performed in order to separate these factors: in these the time for which the radiophosphorus was allowed to circulate was reduced to 1–6 min. The results obtained are given in Table 4.

Table 4 shows that when the time the ^{32}P circulates is short the time available for ^{32}P accumulation is insufficient, and a considerable drop in the absolute counting rate of the fresh organs is observed, as well as a drop in the permeability.

Table 4. Distribution of radioactive phosphorus in the organs in the first and third days after irradiation

Tissue	1 day*		3 days*		1 day†		3 days†	
	Counts/mg tissue	As per cent of activity of blood taken simultaneously	Counts/mg tissue	As per cent of activity of blood taken simultaneously	Counts/mg tissue	As per cent of activity of blood taken simultaneously	Counts/mg tissue	As per cent of activity of blood taken simultaneously
Irradiated:								
Blood	60·1	—	53·5	—	19·6	—	26·7	—
Brain	1·34	2·2	0·6	1·1	0·53	2·7	0·57	2·1
Liver	30·9	51	18	33·8	47·8	243·8	24·2	90·6
Muscle	7·57	13·8	2·55	4·7	5·6	26	3·2	11·9
Control:								
Blood	53·6	—	—	—	22	—	—	—
Brain	1·2	2·2	—	—	0·63	2·8	—	—
Liver	21·4	39·9	—	—	28·7	130	—	—
Muscle	6·66	12·4	—	—	6	27	—	—

* ^{32}P injected 1 min before decapitation.
† ^{32}P injected 6 min before decapitation.

Incorporation of ^{32}P into high-molecular weight compounds in the organs of irradiated and non-irradiated animals

To elucidate the metabolic processes involved in the accumulation of radiophosphorus by the organs, some special experiments were carried out on the rate of ^{32}P incorporation into the acid-insoluble phosphorus fraction. The fractional incorporation was expressed as the ratio between the specific activities of the acid-insoluble and acid-soluble phosphorus fractions (Fig. 3). This shows that the rates of ^{32}P

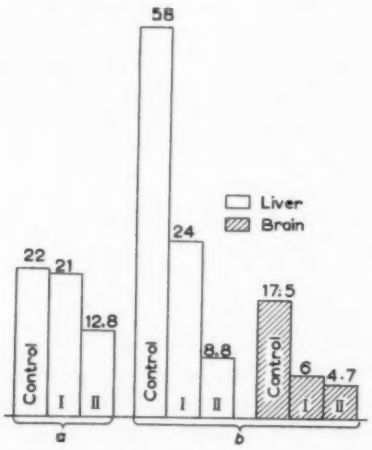

Fig. 3. Effect of X-ray irradiation on the rate of ^{32}P incorporation into the acid-insoluble tissue phosphorus at various times after irradiation.

incorporation into the acid-insoluble phosphorus fractions of both brain and liver were reduced after irradiation. This drop was particularly marked after three days.

Thus our data enable us to assume that the accumulation in the organs on the third day is not due, as might be expected, to the synthesis of high molecular weight compounds insoluble in acid. It must therefore be supposed that the raised ^{32}P accumulation is mainly due to increased organ absorptivity. Several papers (Pasynskii [19], Kuzin *et al.* [20]) indicate an increased organ adsorptivity after ionizing radiation action.

^{131}I distribution in organs of non-irradiated and irradiated animals

Similar results on the isotope distribution ratios in organs of non-irradiated and irradiated animals were obtained by using ^{131}I as tracer.

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TABLE 5. DISTRIBUTION OF ^{131}I IN THE ORGANS OF NON-IRRADIATED ANIMALS WHEN IT IS ALLOWED TO CIRCULATE FOR VARIOUS TIMES (AS PER CENT OF ACTIVITY OF BLOOD TAKEN SIMULTANEOUSLY)

Iodine circulation time (min)	No. of experiments	Brain	Muscle	Liver
1	8	2·8	9·67	32·6
30	16	2·9	11	39·6
120	4	2·4	12·8	53·7

Shtern *et al.* [21] have shown that iodine injected as NaI into the circulation does not pass through the blood-brain barrier. When ^{131}I was used for permeability studies, activity was found in the cerebrospinal fluid, but the authors remark that its penetration there was exceptionally slow. Greenberg *et al.* [12] point out that, of all the ions they studied (K, Na, P, I, Rb, Sn, Br) the blood-brain barrier permeability was the lowest of all for iodine.

In our experiments we could detect only a very small transfer of iodine from blood to brain, and also to liver and muscle in both control and irradiated animals. The data are given in Tables 5 and 6. The radioactive iodine was injected immediately after irradiation, as in the case of phosphorus.

TABLE 6. EFFECT OF IRRADIATION ON THE ^{131}I DISTRIBUTION IN ORGANS (DATA EXPRESSED AS PER CENT OF ACTIVITY OF BLOOD TAKEN SIMULTANEOUSLY)

No. of animals		Brain		Muscle		Liver	
Irradiated	Non-Irradiated	Experimental	Control	Experimental	Control	Experimental	Control
6	16	48	2·9	18	11	44·7	39·6

Quantitative determination of the brain blood content

When the barrier states in the early period after isotope injection are studied, i.e. when the amounts present in the organs are low and the blood activity is high,

the residual blood in the organs may be a source of error. This is particularly so when estimating blood-brain barrier penetration, since the activity of brain tissue rises exceptionally slowly. Table 1 shows that the brain activity during the first 15 min after injecting the phosphorus is 2·2–3·1 per cent of that of the blood, for both control and irradiated animals. This brain activity is also found at 2 hr after injecting radio-iodine into non-irradiated animals (see Table 5). The tissue activities in muscle and liver rise gradually during this period (Table 1).

These data lead one to suppose that the brain activity in the first 15 min after injecting ^{32}P, and in the first 2 hr in the case of ^{131}I, may be explained by residual blood in the brain tissue preparations.

When the ^{131}I was injected 1 min before decapitation the brain activity was found to be the same (2·8 per cent of that in blood) as at 2 hr after injection (2·4 per cent).

Experiments in which the brain was rinsed out 1 min after injecting ^{131}I also indicate that the brain activity is only due to residual blood.

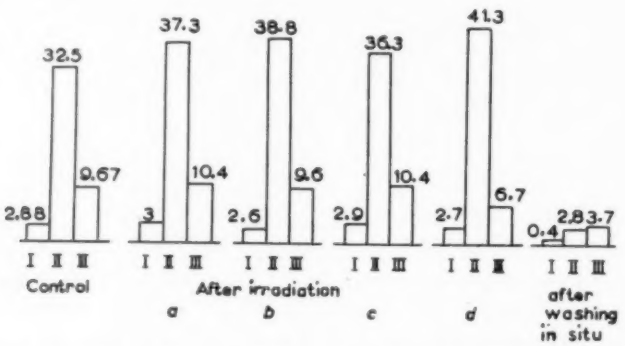

Fig. 4. Residual blood in organs according to ^{131}I (iodine circulation time 1 min). I—brain, II—liver, III—muscle. a—after 3 min; b—after 30 min; c—after 60 min; d—after 120 min. Data expressed as per cent of blood taken at the same time.

The activity of a brain, which had been flushed using a cannula inserted in the aorta and directed towards the head, was some 0·4 per cent of that shown by the blood taken before the washing began. It is of interest that the thyroid activity under these conditions was on the average 2·715 per cent of the blood activity.

The figure for residual blood in the brain derived from our radio-iodine experiments is somewhat higher than that found by Vladimirov [16] with iron: this discrepancy may be due to the iodine being mostly in the plasma, while the iron is in the erythrocytes. The plasma/erythrocyte ratio in the capillaries changes on draining off the blood, the plasma content rising, while the erythrocyte content falls [22–24].

To elucidate effects due to possible haemodynamic changes in the residual blood in the brain after irradiation, the iodine was injected at various times after irradiation, 1 min before decapitation. Fig. 4 shows that the residual blood was almost unchanged at 3, 30, 60 and 120 min after irradiation.

A question remaining unsolved is to what degree the activities of liver and muscle in control and irradiated animals at 1 min after iodine injection are due to residual blood, and to what degree to the iodine penetrating the organs. Preliminary data

show that on flushing the livers and rear limb muscles via the coeliac aorta, or of flushing out the whole animal via the jugular vein, the tissue activities dropped considerably (liver from 32·5 to 2·8 per cent, and muscle from 9·67 to 3·7 per cent). The liver and muscle were not completely freed of blood in these experiments.

SUMMARY

(1) The blood-tissue barrier permeabilities in white rats were studied by injecting radioisotopes, and were found to differ in the various organs of normal animals. When radioactive phosphorus and iodine were used, the greatest permeability was found in liver, and the least in brain.

(2) Single-shot whole-body irradiation of 800 r causes barrier permeability changes. Increased permeabilities were observed in the first day after irradiation, and a sharp drop on the second and third days.

(3) The barrier permeabilities rise during the first day after irradiation: in liver the rise is during the first minute, for muscle during the first 15 min and for brain the first 45 min.

(4) Single-shot whole-body irradiation causes a fall in the incorporation of phosphorus into high molecular weight compounds even during the first hours.

(5) The changes in the functional states of the blood-tissue barriers are due to a number of different disturbances caused by the X-rays.

Translated by J. E. S. Bradley

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CONDITIONED REFLEX ACTIVITY IN DOGS UNDER LOCAL INFLUENCE OF A V.H.F. FIELD UPON CERTAIN ZONES OF THE CEREBRAL CORTEX

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(*Received* 12 *July* 1956)

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A STUDY of the biological action of physical agents used in modern technology is of great importance as a biological problem relating to adaptation to new conditions in the surroundings as well as an important practical scientific problem. The action of electromagnetic fields of very high frequency (V.H.F.) on the central nervous system is of great interest as this kind of energy is deep-acting and may directly influence the nervous centres. Although even in the early work on the biological action of this agent [1–8, etc.] there was no doubt as to the high V.H.F. sensitivity of the nervous system, little work has been done on the action of V.H.F. on cortical functions. We know of only two papers on the influence of V.H.F. upon conditioned reflexes in animals [1, 9] in both of which the V.H.F. was administered to the whole head of the animal. The aim of this paper is to study the influence of local V.H.F. upon separate cortex zones and upon the higher nervous activity of animals.

METHODS AND A SHORT DESCRIPTION OF THE EXPERIMENTAL ANIMALS

As V.H.F. sources we used G.I.F. 250 generators (250 W) and a 50 W generator constructed by E. V. Terent'eva. The frequency was 50 Mc/s. We used a UVCh-TZ unit and special local dose electrodes designed by Terent'eva and Terent'ev [10, 11] to apply the V.H.F.

In all experiments but one we used a ring electrode. The energized electrode is an aluminium strip of 24 mm diameter and the earthed electrode is one of 45 mm diameter surrounding the other, and joined via a continuous aluminium cone with the earthed cable-outer.

Both electrodes were enclosed in an insulating layer of Perspex of thickness 2·5 mm under the energized electrode.

The insulator was of a greater diameter than the metallic parts, and was fixed to the shaved skin of a suitable part of the dog's head with Mendeleev's putty placed round the projecting end: it was held by a hand on the cable.

One experiment upon the frontal lobes was carried out with slightly bent brass strip of 24 mm diameter enclosed in Perspex as driven electrode, the other being in the form of a metal grid 12×14 cm joined to the metal braiding on the cable and fixed to the animal's lower jaw.

The V.H.F. was administered outside the chamber prior to the conditioned reflex experiment. Before the V.H.F. experiments the dogs were accustomed to the

surroundings and to the procedure used by attaching the electrodes and switching on the generator without energizing the valve anode. V.H.F. fields of low, medium and high intensities were used.

A weak field is one in which the output (administered in the normal fashion) was 7–14 W lasting for about 5 min. This caused no restlessness. Medium intensities (20–25 W) caused defensive reactions. Strong fields involved outputs of 40–50 W lasting 10 min. There was no need to bind the dogs when high intensities were used. It was easy to keep them in the necessary position by holding their collars or simply by telling them to stand quietly.

Four dogs were studied via their conditioned salivary reflexes to food.

Short description of the animals

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I. Zheltyi, male, 16 Kg in weight, 2 or 3 years old when the work began. Type of nervous system being strong, balanced with a certain weakness of mobility of the nervous processes. Stereotypes to the following stimuli were developed:

(1) Offering food (without conditioning stimulus).
(2) 100 metronome ticks per min (metronome 100).
(3) Light (switching on a 40 W lamp)—a 40 W lamp being also used for general lighting.
(4) Bell of intensity 64 dB (zero level taken as 2×10^{-4} bar).
(5) 60 metronome ticks per min (metronome 60) as differentiating stimulus.
(6) Apparatus for slightly touching the animal's skin at the thigh (touching apparatus +positive conditioned stimulus).
(7) Touching apparatus on the animal's side (a touching apparatus—) as differentiating stimulus.
(8) 100 metronome ticks per min.

II. Borzik, male, 12 Kg, about 2 years old at the beginning. Type of nervous system: strong but unbalanced. In this dog we worked out the same positive conditioned reflexes as in Zheltyi, but in view of the unbalanced character of his nervous processes we included only rather easy differentiation into the stereotype: 52 ticks of the metronome per min (to be differentiated from 100) which, however, even after 240 repetitions still remained a relative one.

III. Saltan, a big male, 20 Kg, 4–5 years old at the beginning. Type of nervous system: strong, unbalanced with a low mobility of nervous processes. With Saltan as well as with Borzik we excluded all tactile differentiations, but there was no need to make the metronome differentiations easier for him.

IV. Druzhok, male, 9 Kg, 2 years old at the beginning. His conditioned reflex background was very unstable. Disorders of his higher nervous activity (HNA) were observed during which periods the conditioned reflexes became suddenly lower without any obvious reason and the motor reaction on the presentation of the food bowl after several stimulations began to be late or to be absent. Because of this unstable character of its conditioned reflex background we did not try to establish the type of HNA in this dog. However, having observed his conditioned reflex activity further for a long time, and after having tried the effect of bromide and caffeine, we concluded that this dog had a weak type of HNA.

EFFECT OF A V.H.F. FIELD ON THE AUDITORY AREAS OF THE CORTEX

In order to produce a bilateral action on the auditory areas of the cortex we used the apparatus designed by E. V. Terent'eva. The V.H.F. field was transmitted from a dosimeter over a T-junction leading to two symmetric cables going to two ring-shaped electrodes affixed on either side to the shaved skin of the skull in the region corresponding to the projection of the temporal lobes of the cerebral cortex.

A bi-temporal application of the electrodes has been used, as is well known, by a series of experimental and clinical investigations in order to act with a V.H.F. field on the diencephalon.

The difference between our method and the method generally used is that by virtue of the construction of the ring-shaped electrodes in our case there is no transcerebral effect but only a tangential effect on the auditory areas of the cortex.

In dog Zheltyi the first administration of a V.H.F. field of power 10 W caused a lowering of the conditioned reflex to light.

This was an isolated case which did not re-occur in any of the dogs.

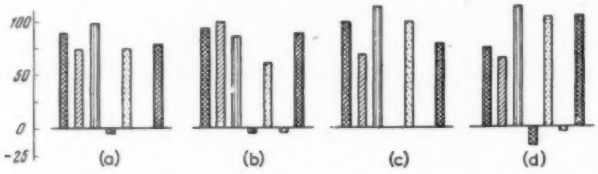

FIG. 1. Deterioration of auditory discrimination following the bilateral administration of V.H.F. fields to the auditory areas of the cortex. Dog Zheltyi.
(a) and (b)—experiment 293, 13 June 1949, and experiment 294, 15 June 1949—normal; (c) experiment 295, 16 June 1949, immediately after bilateral administration of V.H.F. field, strength 20 W, during 5 min, to the auditory areas of the cortex; (d) experiment 296, 17 June 1949—the second day after the administration of V.H.F. field. Above abscissa: positive conditioned reflexes; columns cross-hatched—metronome 100; columns with diagonal shading—effect of light; columns with vertical shading—bell; stippled columns—effect of tactile instrument on thigh.
Below abscissa: discriminations; columns cross-hatched—metronome 60 (for Borzik and Druzhok-metronome 52); stippled columns—tactile instrument on the flank.

Administration of a 15 W V.H.F. field carried out twice on the auditory areas did not produce any changes in the conditioned reflex activity.

The administration of a 20 W V.H.F. field did not cause any changes in the conditioned reflexes on the day of the administration, but on the following day there was a deterioration of discrimination in the auditory analysor, whereas the tactile discrimination did not suffer at all. On the third day the normal conditions returned (Fig. 1). Although the deterioration of auditory discrimination was of slight degree only, it was an unusual event for this dog; for this animal both discriminations were absolute. Administration of a 38 W V.H.F. field on the second day after the administration evoked a lowering of the conditioned reflexes to auditory stimuli. On the third day the conditioned reflexes had returned to the normal level, but the deterioration of discrimination could again be observed in the auditory analysor only.

For the period of a month this dog was not subjected to the action of the V.H.F. fields. When a month later a 24 W V.H.F. field was administered to the auditory areas of the cortex, this caused no changes whatsoever in conditioned reflex activity.

In the dog Borzik we administered 3 doses of 12–14 W. In two cases this caused no effect whatsoever, but in one case we observed on the day of the administration lowering of the conditioned reflexes to the metronome without any improvement in the discrimination (Fig. 2). Borzik never had an absolute discrimination and the discriminatory stimulus evoked salivation even in normal conditions.

Administration of 21 and 50 W V.H.F. fields caused deterioration of discrimination. The salivation evoked by the discriminatory stimulus was much higher than the normal variations. The deterioration of discrimination was more marked in the case of the administration of the stronger dose (Fig. 3).

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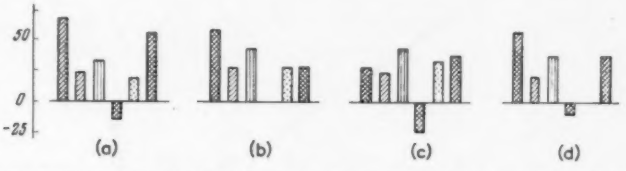

FIG. 2. Deterioration of conditioned reflex to metronome following bilateral administration of a V.H.F. field to the auditory areas of the cortex. Dog Borzik.
(a) and (b) experiment 199, 18 July 1949, and experiment 201, 20 July 1949—initial background; (c) experiment 202, 22 July 1949, immediately after bilateral administration of a 12 W V.H.F. field, duration 5 min, to the auditory areas of the cortex; (d) experiment 203, 23 July 1949, second day after administration.

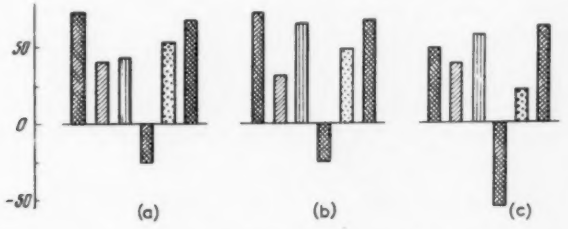

FIG. 3. Deterioration of discrimination following bilateral administration of a V.H.F. field to the auditory areas of the cortex. Dog Borzik.
(a) and (b) experiment 239, 21 October 1949, and experiment 242, 25 October 1949, initial background; (c) experiment 243, 26 October 1949, immediately after alternate exposure of both auditory areas of the cortex to a 55 W V.H.F. field—duration 10 min.
Columns cross-hatched below abscissa—discrimination, metronome 52. Remaining captions as in Fig. 1.

Unfortunately in this dog we had worked out only a metronome discrimination, which deprived us of the possibility to check the state of other analysors.

Unilateral exposure of the right auditory area of the cortex to a 55 W V.H.F. field for 10 min had no effect whatsoever, although the power of the field absorbed by the animal was larger than in the case of a bilateral exposure to a dose of 21 W.

Repeated bilateral administration of a dose of 50–55 W caused only a slight decrease in the auditory conditioned reflexes, which did not go below the lower limit of normal variations.

Thus in Borzik and Zheltyi, exposure to strong V.H.F. fields led to a decrease of the re-activity of the cortex to V.H.F.

Saltan was distinguished by a high resistence towards V.H.F. but after a course

of exposures to high doses of V.H.F. on the cerebellum and on the hind-half of the hemispheres there was a temporary elevation of the re-activity of the cortex to the actions of V.H.F.* This was the background on which we carried out two experiments on the actions on the auditory areas of the cortex of 23–28 W V.H.F. fields with exposure of 10–12 min. In both cases we observed a decrease of conditioned reflexes only to the metronome, whereas the conditioned reflexes relative to other analysors showed no change at all. In one of these experiments the changes in the conditioned reflex activity appeared on the day of administration of the dose. In the other experiment a reaction of the same character took place on the second day following the administration of V.H.F. One of these experiments is illustrated in Fig. 4.

Two-and-a-half months later, after the normal re-activity V.H.F. had been restored in the dog, the same exposure caused no change whatsoever.

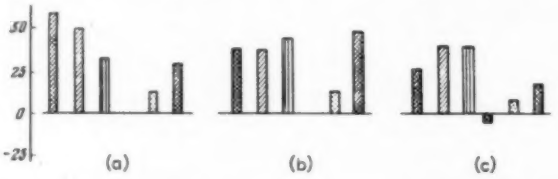

FIG. 4. Decrease of conditioned reflex to metronome after bilateral exposure of the auditory areas of the cortex. Dog Saltan.
(a) experiment 241, 2 June 1949, initial background; (b) experiment 242, 3 June 1949—immediately after bilateral administration of 28 W V.H.F. field, exposure 10 min on the auditory areas of the cortex; (c) experiment 243, 4 June 1949. Second day after exposure to V.H.F. Captions as in Fig. 1.

It thus appears that we exposed the auditory areas of the cortex bilaterally 15 times in 3 dogs belonging to the strong type. Marked changes in the conditioned reflex activity were observed in 8 cases. In the result of 7 of these 8 exposures that had shown an effect, the changes in the conditioned reflexes were limited to the auditory analysor.

Quite another picture was observed in the dog belonging to the weak type—Druzhok.

As was said before, the conditioned reflex background in this dog was characterized by the lack of stability. Often the motor feeding reaction after giving the food bowl was delayed and frequently he refused the food—mostly towards the end of the experiment. On this background a bilateral exposure of the auditory areas of the cortex caused a general weakening of the excitation processes in the auditory as well as in the optic analysors.

There was not only a decrease of the artificial conditioned reflexes but the dog refused the food more frequently than before.

These data are summarized in Table 1.

From Table 1 it can be seen that on the day of exposure of both auditory areas of the cortex, as well as on the following day, there were no changes in the conditioned reflex activity; on the third day after the procedure, however, a decrease of the conditioned reflexes was observed.

The disorders of the conditioned reflex activity reached their maximum on the

* Experiments with the effects on the cerebellum will be published separately.

sixth day after the exposure (Experiment 340), after which the dog returned to the initial background.

Unilateral exposure of the left auditory area of the cortex to an 8 W V.H.F. field caused in this dog also a decrease of conditioned reflexes from various analysors (Table 2).

The changes of the conditioned reflexes following the unilateral action on the left

TABLE 1. INFLUENCE OF BILATERAL EXPOSURE OF THE AUDITORY AREAS OF THE CORTEX ON CONDITIONED REFLEX ACTIVITY.* DOG CALLED DRUZHOK (1949)

Stimulus	No. and date of exp.		Before exp. 335, 15 August, there was a bilateral exposure of the auditory areas of the cortex to 20 W V.H.F. for 10 min					
	11 Aug. 332	13 Aug. 334	15 Aug. 335	16 Aug. 336	17 Aug. 337	18 Aug. 338	20 Aug. 340	21 Aug. 341
Metronome 100	27·5	37·5	32·5	15·0	17·5	16	4·0 refused food	30·0
Light	7·5	13·5	10·0	10·0	4·0	8·5	8·5 refused food	32·5
Bell	17·5	35·0	2·5	19·0	4·0	9·0	0 refused food	20·0
Metronome 52 Discrimination	0	2·5	2·5	0	4·0	4·0	0	14·0
Light	0	0	0	3·5	0	0	10·0	14·0
Metronome 100	13·5 refused food	9·0 refused food	12·5 refused food	3·5 refused food	5·0 refused food	0	15·0 —	17·5 —

* The salivation is here, as well as everywhere else in this paper, expressed in scale divisions: 1 drop = 4 divisions.

TABLE 2. INFLUENCE ON CONDITIONED REFLEX ACTIVITY OF UNILATERAL EXPOSURE OF THE AUDITORY AREAS OF THE CORTEX TO A V.H.F. FIELD. DOG DRUZHOK (1950)

Stimulus	No. and date of exp.		Before exp. 453, 30 October, there was an exposure of the left auditory area for 5 min to a 7–10 W V.H.F. field		
	26 October 450	27 October 451	30 October 453	31 October 454	1 October 455
Metronome 100	35·0	32·5	5·0	5·0	18·0
Light	25·0	10·0	4·0	4·0	4·0
Bell	26·0	20·0	12·5	15·0	0
Metronome 52	9·0	0	0	3·5	3·5
Light	9·0	10·0	4·0	3·5	4·0 refused food
Metronome 100	15·0 refused food	15·0 refused food	15·0 —	4·0 refused food	9·0 refused food

auditory area were less marked than after bilateral action, but in this case too they were not limited to the auditory analysors only.

It must be taken into account that in this case of unilateral action the dose of V.H.F. was considerably smaller than with bilateral action.

ACTION OF A V.H.F. FIELD ON THE FRONTAL LOBES

For the action on the frontal lobes of the cerebral cortex we used a single ring-shaped electrode which was fixed on the skull in the area corresponding to the projection of the frontal lobes, in such a way that the centre of the electrode was exactly in the median line. Both frontal lobes were acted on simultaneously. It could not be excluded that other parts of the cortex situated in the anterior halves of the hemispheres were exposed to the V.H.F. field, especially the cortical part of the motor analysor.

In one of the experiments we used an active electrode brass disc and as the indifferent electrode the metallic net described on page 198.

The frontal lobes of the cortex of dog Zheltyi were exposed three times to a V.H.F. field; twice to a 10 W field and once to a 22 W field, with a duration of 5 min. Changes of the conditioned reflex activity were observed in two cases on the day of exposure or on the next day. These changes affected conditioned reflexes related to various analysors. In one case there was a decrease in the conditioned reflexes to the metronome and to the tactile instrument and in the other experiments there was a deterioration of discrimination in the auditory as well as in the cutaneous analysor (Fig. 5).

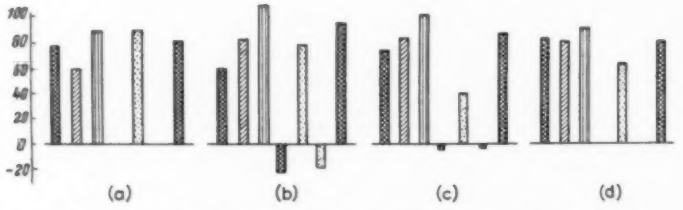

Fig. 5. Deterioration of discriminations and decrease of conditioned reflexes in the auditory and cutaneous analysors after exposure of the frontal lobes of the cortex to a V.H.F. field. Dog Zheltyi.
(a) experiment 273, 21 May 1949, initial background; (b) experiment 274, 23 May 1949, immediately after the exposure of the frontal lobes of the cortex to a V.H.F. field; (c) experiment 275, 24 May 1949; (d) experiment 276, 25 May 1949. Captions as in Fig. 1.

Dog Borzik was exposed twice—once to a 14 W field and the second time to a 20 W field, each exposure lasting 5 min; no changes in his conditioned reflex activity were observed at all.

Above we pointed out that in the exposure of the auditory areas to V.H.F. no effect was observed in any of the cases. We will return to this question in the discussion of our results.

In dog Druzhok the frontal lobes were exposed twice to a 20 W field, the exposure lasting 10 min. In both experiments this exposure had an exceptionally strong depressing influence on the conditioned reflex activity. In one case the reaction could be observed on the second day, in the second case on the third day after the

TABLE 3. INFLUENCE ON THE CONDITIONED REFLEX ACTIVITY OF EXPOSURE OF THE FRONTAL LOBES TO A V.H.F. FIELD. DOG DRUZHOK (1949)

Stimulus	No. and date of exp.	Before exp. 293, 11 May, there was an exposure of the frontal lobes for 10 min to a 20 W V.H.F. field		
	10 May 292	11 May 293	12 May 294	13 May 295
Metronome 100	47·5	0	5·0 refused food	30·0
Light	22·5	0	0 refused food	25·0
Bell	25·0	30·0	10·0 refused food	3·5
Metronome 52	4·0	0	0	4·0
Light	15·0	0	0 refused food	4·0
Metronome 100	10·0	17·5	0 refused food	10·0 refused food

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exposure. In this dog the artificial as well as the natural conditioned feeding reflexes proved to be inhibited during the experiment. The dog showed no reaction when the food bowl was given and refused food for the whole duration of the experiment. The results of one of these experiments are presented in Table 3.

In another case after the exposure of the frontal lobes a pathological behaviour of the dog was observed during the experiment. The dog was restless, tore off the capsule used for the collection of saliva, and tried to escape from the stand.

A general review of the experiments on all the dogs is presented in Table 4.

DISCUSSION OF RESULTS

As can be seen from Table 4, we carried out 25 experiments on 4 dogs, exposing individual areas of the cerebral cortex to a V.H.F. field. Marked changes in the higher nervous activity were observed in 15 cases.

The cortical reaction to the action of V.H.F. shows a long latency. Only in 6 experiments out of 15 were changes in the conditioned reflexes observed immediately after the procedure. In the majority of cases, however, the reaction appeared on the second, sometimes on the third and fourth days. The disorders in the conditioned reflex activity continued for a period of one or two days, after which the activity returned to the original background.

The reactivity of the cortex to the action of V.H.F. shows extreme variations. In some of the experiments certain doses evoked marked changes in the conditioned reflexes whereas in other experiments the same doses showed no effect whatsoever. The unstable character of the reactivity of the organism to V.H.F. was described long ago in the literature [2, 3, 4, 5, 7 and many others]. In spite of much experimental and clinical material the causes for the variations in reactivity of the organism to V.H.F. are still not clear. Our experiments show that the reactivity of the cortex to these influences is also very unstable.

Comparing the results of the action of V.H.F. on the auditory areas of the cortex and on the anterior halves of the cerebral hemispheres in different dogs, it can be

TABLE 4

No.	Type of electrode	Dose	Name of dog	Date and No. of exp.	Locus of action	Time of beginning of changes in conditioned reflexes (C.R.)	Nature of effect
1	Ring-shaped	10 W 5 min	Zheltyi	30 May 1949 341	Both auditory areas	On day of exp.	Decrease of C.R. to light
2	Ring-shaped	15 W 5 min	Zheltyi	2 June 1949 344	Both auditory areas	—	No changes in C.R.
3	Ring-shaped	20 W 5 min	Zheltyi	16 June 1949 356	Both auditory areas	2nd day after exp. Normal on 3rd day	Deteriorn. of discrimn. in auditory analysor only
4	Ring-shaped	13–16 W 5 min	Zheltyi	5 September 1949 383	Both auditory areas	—	No changes in C.R.
5	Ring-shaped	38 W 10 min	Zheltyi	28 September 1949 402	Both auditory areas	2nd day after exp.	2nd day decrease of C.R. to auditory stimuli in 1st half of exp. 3rd day deteriorn. of discrimn. in auditory analysor
6	Ring-shaped	25 W 10 min	Zheltyi	1 November 1949 415	Both auditory areas	—	No changes in C.R.
7	Ring-shaped	14 W 6 min	Borzik	6 June 1949 171	Both auditory areas	—	No changes in C.R.
8	Ring-shaped	21 W 6 min	Borzik	30 June 1949 182	Both auditory areas	On day of exp. Normal on 2nd day	Deteriorn. of discrimn. (in this dog aud. discrimn. only had been estabId.
9	Ring-shaped	12 W 5 min	Borzik	22 March 1949 203	Both auditory areas	On day of exp. Normal on 2nd day	Decrease of C.R. to metronome
10	Ring-shaped	12 W 5 min	Borzik	8 September 1949 217	Both auditory areas	—	No changes in C.R.
11	Ring-shaped	55 W 10 min	Borzik	28 September 1949 232	Right auditory area	—	No changes in C.R.
12	Ring-shaped	50–55 W 10 min	Borzik	26 October 1949 243	Both auditory areas	On day of exp. Normal next day	Break-down of discrimn. (in this dog auditory discrimn. only had been estabId.)
13	Ring-shaped	50 W 10 min	Borzik	3 November 1949 249	Both auditory areas	—	Very small decrease of C.R. to auditory stimuli
14	Ring-shaped	25 W 10 min	Saltan	31 May 1949 239	Both auditory areas	On day of exp. Normal next day	Decrease of C.R. to auditory stimuli only
15	Ring-shaped	28 W 10 min	Saltan	3 June 1949 242	Both auditory areas	2nd day after exp. Normal on 3rd day	Decrease of C.R. to metronome only
16	Ring-shaped	23 W 10 min	Saltan	22 August 1949 270	Both auditory areas	—	No changes in C.R.
17	Ring-shaped	20 W 10 min	Druzhok	15 August 1949 335	Both auditory areas	3rd day after exp. Unstable background	Disappearance of C.R. to all stim. except 1st. Delay in feeding from food bowl
18	Ring-shaped	7–8 W 5 min	Druzhok	30 October 1950 453	Left auditory area	On day of exp. Maximal changes on 2nd and 3rd day	General decrease of C.R.
19	Act. electrode-plate. Indifferent electrode metallic network	9–10 W 5 min	Zheltyi	20 April 1949 316	Frontal lobes	On 2nd day after exp. Normal on 4th day	Decrease of C.R. to metronome and to tactile instrum.
20	Ring-shaped	22 W 5 min	Zheltyi	23 May 1949 335	Frontal lobes	On day of exp. Normal on 3rd day	Auditory on day of exp. deteriorn. of discrimn. (tactile). On 2nd day decrease of C.R. to tactile instrum.
21	Ring-shaped	10–12 W 5 min	Zheltyi	25 August 1949 374	Frontal lobes	2nd day after exp. Normal on 5th day	Deteriorn. of discrimn. (auditory and tactile)
22	Ring-shaped	14 W 5 min	Borzik	23 May 1949 161	Frontal lobes	2nd day after exp. Normal on 5th day	No changes in C.R.
23	Ring-shaped	20 W 5 min	Borzik	6 July 1949 187	Frontal lobes	—	No changes in C.R.
24	Ring-shaped	20 W 10 min	Druzhok	11 May 1949 293	Frontal lobes	2nd day after exp. Normal 3rd day	Disappearance of all C.R., refuses food from food bowl
25	Ring-shaped	23 W 10 min	Druzhok	5 July 1949 325	Frontal lobes	3rd day after exp. Normal on 4th and 5th day	Disappearance of all C.R., refuses food from food bowl

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seen that the cortical reaction depends on the localization of action as well as on the typological peculiarities of the animal.

In dogs with a strong type of higher nervous activity (Zheltyi, Borzik, Saltan) bilateral exposure to V.H.F. caused, in 7 experiments out of 8 that gave an effect, changes in the auditory analysor only. In some of the experiments, the excitatory processes, in others the inhibitory processes, mainly suffered. In one case there was a decrease of positive conditioned reflexes in the first phase and a deterioration of discrimination in the second phase. A decrease of the conditioned reflexes to auditory stimuli and disorders of the auditory discrimination have been observed, as is well known, at certain periods after partial or complete removal of the auditory areas [12–16]. On this basis it could be presumed that we managed to administer an influence of relatively localized character upon the cortical areas in question. In one of the experiments on dog Zheltyi after the action on the auditory areas there was a decrease in the conditioned reflex to visual stimuli. In the literature, cases are described of disorder in conditioned reflexes from the visual analysor after operations on the temporal lobes of the cortex [12, 14, 16]. In a dog of strong type (Borzik) unilateral action of V.H.F. on one of the auditory areas of the cortex caused no changes whatsoever in the conditioned reflex activity, in spite of the fact that the dose administered was higher than in the case of bilateral action. Action on the anterior halves of the cerebral hemispheres in a dog of the strong type (Zheltyi) in three instances caused changes in the conditioned reflexes from various analysors. We have at present not sufficient material to explain the peculiarities of the reactions of a strong type of dog to the actions of V.H.F. on the frontal lobes of the cortex, but we may mention the dependence of the reaction on the localization of the action. This dependence suggests that reflexes from the skin and muscles and general changes of the cerebral blood circulation do not play a decisive part in the effects observed by us, and the main factors were changes of the functional state of the parts of the brain subjected to the action. It is difficult at present to answer the question whether the changes in the conditioned reflex activity are a result of primary action of V.H.F. on nervous tissues, or rather a secondary result of local circulatory disorders. It seems most probable that both factors are concerned, as it is well known that cerebral vessels are extremely sensitive to exposure to V.H.F. and, on the other hand, brain tissue has a high absorption coefficient for V.H.F. [17].

In the case of bilateral action of V.H.F. on the temporal lobes of the cortex in dogs of a strong type we did not observe the phase of general disorders in the conditioned reflex activity, which usually occurs after the removal of any part of the cortex—as Pavlov described it, "the echo of the blow caused by the operation over the whole mass of the hemispheres" [18]. It is obvious that our procedures, even with a high power field, were not severe, which is suggested also by the relatively quick return to normal conditions within two or three days.

In opposition to the observations on dogs of a strong type of HNA, a dog of a weak type (Druzhok) showed, after exposure of the auditory areas of the cortex to V.H.F., general disorders of higher nervous activity. The same disorders appeared in a lesser degree after unilateral action on the left temporal region. This suggests the significance of the typological peculiarities of the animal in the cortical reactions to exposure to V.H.F.

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CONCLUSIONS

(1) The action of 7–55 W V.H.F. fields on individual regions of the cortex of dogs causes reversible changes of conditioned reflex activity. A decrease in positive conditioned reflexes was observed in certain cases, and a deterioration of discrimination in others. In two experiments, the decrease of the conditioned reflexes and the deterioration of discrimination were observed at various phases after a single exposure to V.H.F.

(2) The reactivity of the cortex to V.H.F. fields is subject to sharp variations. Doses that in certain cases bring about considerable changes in conditioned reflex activity are in other cases ineffective.

(3) The reactions of the cortex to V.H.F. fields depend on the localization of the action and on the typological peculiarities of the animal.

(4) After bilateral action on the auditory areas of three dogs of strong type of HNA, in 7 experiments out of 8 the changes in conditioned reflex activity were related to the auditory analysor only.

In the case of weak-type dogs, action on the auditory areas of the cortex (both bilateral and unilateral) brought about a decrease in conditioned reflexes both from the auditory and the visual analysors. At the same time, cases of lag in the motor feeding reaction and of refusing food became more frequent.

(5) Action on the frontal lobes of the dogs of a strong type of HNA and of those of a weak type caused changes in the conditioned reflex activity affecting different analysors (visual and auditory, auditory and cutaneous).

Translated by F. S. FREISINGER

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USE OF IONIZING RADIATION AGAINST PESTS OF STORED GRAIN

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(*Received* 1 *August* 1956)

The effect of ionizing radiations on various insects has been studied from many aspects by many workers. One of the general conclusions to be drawn from this work is that insects have a high resistance to the lethal action of ionizing radiations, and that, conversely, the reproductive organs are very sensitive to their mutagenic action. Doses of several thousand roentgens are required to sterilize insects. The various forms, genera, families, and orders of insects appear of comparable sensitivity to ionizing radiations. This gives us a method of dealing with insects which infest any product on prolonged storage based on ionizing radiation.

There have been some fragmentary attempts to explain the effects of radium [1] and X-rays [2] on the reactions of some insects representative of those infesting flour and grain. It is clear, however, that these could not be used to develop a suitable method, since neither radium preparations nor X-ray equipment are convenient means of delivering a high dose of radiation to infested grain bodies in a short time. In recent years, however, fresh studies dealing with developments in radiation pest control have appeared [3–13]: due to the development of nuclear technology these represent a real opportunity for effective action. All the same these papers are incomplete, and are inadequate for creating and using the new facility in practice. The similarity between the actions of X-rays, γ-rays and fast electrons on insects reported here enabled us to carry out further experiments with X-rays, these being most conveniently produced: there is then no further obstacle to developing methods of pest control which use any such ionizing radiations.

MATERIALS AND METHODS

The most troublesome pest of stored grain, *Calandra oryzae* L., (rice weevils) was used in the experiments. Since insects in any stage of development may be present

in the infested material at any given time, in the form of eggs, grubs, cocoons and very young weevils, as well as weevils of all ages, it was necessary to determine the degree of radio-resistance in each developmental stage. We took into account the fact that large radiation doses cannot in practice be used.

We decided to determine the minimum radiation doses required to sterilize the insects completely at any developmental stage. The irradiation was carried out with a RUM-3 X-ray unit (200 kV; filter 0·55 mm Cu) and with a RUP-3 (400 kV; filter 2 mm Cu + 0·25 mm Fe). The doses and dose rates are given in the tables. The necessary stages of pest development could be obtained by adding in advance some 500 mature weevils to each 100 gm portion of grain.

The grain (moisture content 14–14·7 per cent), which had been infested with weevils at various stages of development, was irradiated in triplicate in linen bags. During the irradiation the grain in the bags was stirred 1–3 times, depending on the irradiation time. The temperature during irradiation did not alter by more than 1–3°C. The control series (3–6 specimens of each) were given the same treatment, apart from irradiation.

Each irradiated and control specimen of infested grain was placed in a separate vessel for further studies: the vessels being kept in a thermostatically controlled box at 22–23°C and relative humidity 70·7 per cent.

The development and mortality amongst eggs, grubs and cocoons was studied by periodic removal and X-ray screening of the grain, as well as from the weevil count.

Weevil survival in the various series was determined. The weevils found were placed on fresh layers of grain: by taking a count of the younger-generation weevils the degree of sterilization in each experiment was determined. The yield of second-generation weevils showed the later sterilization consequences of irradiation of the grandparents in the same way.

RESULTS

The results are given in Tables 1–5. Table 1 shows that 3,000 r delivered to the same stages of weevil development prevented completely, or almost completely, their transformation to adult forms. But when the older grubs were irradiated, particularly the pronymphs and young cocoons, some fraction of them attained the weevil

Table 1. Mean number of weevils (as per cent of control) produced after irradiation at various stages of development with different X-ray doses (control = 100)

Stage irradiated, and age in days	Dose, r (rate 250–340 r/min				
	1,000	3,000	5,000	8,000	12,000
Eggs 1–2	5·5	0	0	0	0
1–3	9·0	0	0	0	0
1–6	56·5	1·8	0	0	0
Grubs 1–5	51·0	0	0	0	0
15–20	74·9	0·97	0	0	0
24–29	48·5	16·9	0·5	0	0
Pronymphs and young cocoons	72·0	21·9	36·4	15·1	7·5

TABLE 2. PER CENT OF WEEVILS WHICH DIED AFTER IRRADIATION WITH DIFFERENT X-RAY DOSES AT VARIOUS PREVIOUS STAGES OF DEVELOPMENT*

Stage irradiated, and age in days	Control	Dose, r (dose rate 250–340 r/min)				
		1,000	2,000	5,000	8,000	12,000
Eggs 1–2	0·5/18·3	13·0/78·4	Did not develop			
1–3	0/23·5	7·4/76·9	Did not develop			
1–6	0·03/13·8	8·3/61·6	35·4/1,000	Did not develop		
Grubs 1–5	0/18·0	2·8/66·6	Did not develop			
15–20	0·16/25·1	1·2/72·6	50/100	Did not develop		
24–29	0·35/21·4	20·7/98·9	71·2/100	90·9/100	Did not develop	
Pronymphs and young cocoons	0·4/27·9	9·4/96·8	86·3/99·6	98·3/100	97·4/100	95/100

* The numerator is the number dead on the 18th–26th day after emergence; the denominator is the number of weevils dead at the 61st–76th day after emergence.

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stage at doses of 5,000, 8,000 and even 12,000 r. However, Table 2 shows that most of these then died at 18–26 days after emergence, and almost 100 per cent had died by the sixty-first to the seventy-sixth day (at doses of 1,000 and 3,000 r) or the whole 100 per cent (doses of 5,000, 8,000 and 12,000 r).

When young (1–5 days) or old (2 months) organisms were irradiated, the subsequent deaths occurred more rapidly than in the controls (Table 3). The rate rose with the radiation dose. The old weevils died earlier than the young ones.

All the adult weevils, whether irradiated at this or some earlier stage of development, were provided with normal pairing and oviposition on uninfested grain. But Table 4 shows that even doses of 1,000 r markedly reduced the chances of producing descendents when the irradiation was at any stage of development. Doses of 3,000 r reduced it still more, even to zero. Doses of 5,000, 8,000 and 12,000 r sterilized all

TABLE 3. DEATHS OF WEEVILS IRRADIATED AT VARIOUS LEVELS*

Interval after irradiation (days)	Per cent dead					
	Control	Dose, r (rate 250–340 r/min)				
		1,000	3,000	5,000	8,000	12,000
10	0·7/6·3	2·6/14·3	1·3/44·1	2·6/46·4	2·6/60·5	4·7/65·6
44	2·0/45·8	6·7/63·9	27·3/94·3	95·4/99·6	93·3/100†	100‡/100**
97	59·0/71·5	88·3/96·8	97·9/99·3	100/100	—	—
107	59·0/78·2	89·9/97·9	100/99·8	—	—	—
117	60·4/95·1	96·6/98·9	100/100	—	—	—
135	61·8/97·1	100/100	—	—	—	—
148	62·4/97·1	—	—	—	—	—

* Numerator is for young weevils, denominator for old weevils.
† Ab. day 26. ‡ Ab. day 33. **Ab. day 36.

Table 4. Number of first-generation weevils (as per cent of control) produced by parents irradiated at various stages of development with different X-ray doses (control = 100)

Stage irradiated, and age in days	Dose, r (rate 250–340 r/min)				
	1,000	3,000	5,000	8,000	12,000
Eggs 1–2	28·0	Did not develop			
1–3	34·0	Did not develop			
1–6	50·6	16·0	Did not develop		
Grubs 1–5	32·0	Did not develop			
15–20	12·1	Developed, but gave no descendants			
24–29	27·7	8·3	Developed, but gave no descendants		
Pronymphs and young cocoons	66·5	16·0	Developed, but gave no descendants		
Young weevils	30·9	2·2	0·13	0·13	Gave no descendants
Old weevils	32·1	3·5	0·3	0·008	0·5

Table 5. Mean numbers of generation II (as per cent of control) from irradiated grandparents (control = 100)

Ages of irradiated grandparents	Doses to irradiated grandparents in r (dose rate 250–340 r/min)				
	1,000	3,000	5,000	8,000	12,000
Young weevils (1–5 days)	56·5	50·4	The parents were individual specimens and gave no descendants		
Old weevils (2–3 months)	88·8	51·8	25·9		The parents were individual specimens and gave no descendants

weevils derived from the organisms irradiated at any stage. Only those irradiated as young or old weevils produced a subsequent generation in minute numbers.

When the grandparents were irradiated at 12,000 r as young weevils, this small first generation gave rise to no second generation. But those irradiated at 5,000 r at later stages gave a certain number of second-generation descendents.

CONCLUSIONS

It was found that a dose of 5,000 r of X-rays applied to food grains infested with weevils (*Calandra oryzae* L.) at any stage of development was very close to the absolute sterilizing dose: this value should be used in technological calculations on irradiators designed to disinfest grain on State granary conveyors.

Doses of 8,000–10,000 r may possibly be adequate to sterilize any type of grain and flour pest (although this needs to be verified). It will be possible to use radiation

sterilization on grain pests if the parameters found can be provided in large-scale grain handling. There are many papers dealing with this [14–24].

Preliminary calculations show that it is theoretically and technically possible to design γ-ray units for large-scale sterilization of moving grain.

The type and form of irradiator can only be chosen after the detailed calculations which are in hand have been completed.

Translated by J. E. S. BRADLEY

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ON THE PHOTO-OXIDATION OF THE SULPHYDRYL GROUPS OF DISSOLVED EGG ALBUMIN

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(*Received* 1 *August* 1956)

IT IS already known that under the action of ultra-violet radiation certain constituent amino and groups of dissolved proteins (histidine, trytophane, cystein) can be oxidized or decomposed "selectively" before the protein is transformed into products of photolysis of smaller molecular weight. It has been previously shown that the photo-oxidation of protein is not bound up with, and occurs independently of,

photodenaturation [1]. On the basis of this we suggested that in irradiated protein solutions the part of the protein still capable of denaturation is already partially oxidized.

Later we succeeded in demonstrating spectrophotometrically a changed condition of the tyrosine groups of the irradiated protein long before its complete denaturation [2]. We did not, however, at that time separate the native protein fraction from the irradiated solution; the changes in it were only deduced from a comparison of the course of the denaturation with that of the "freeing" of the tyrosine groups. The measurements were complicated by the presence in the solution of photo-products with powerful absorptions in the ultra-violet. In the experiments described below the "native" fraction of the protein was separated and investigated directly. From the egg albumin solution after irradiation the denatured protein was removed, and the remaining albumin was freed from low-molecular products of the photo-decomposition. In this irradiated "native" fraction the sulphydryl groups oxidizable by iodine were estimated.

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1. SEPARATION OF THE NATIVE PROTEIN FROM THE IRRADIATED SOLUTION, AND ITS PURIFICATION

The whole work was carried out on a solution of purified egg albumin of the same concentration obtained from the egg white of fresh hens' eggs. Globulins were removed by half saturation with ammonium sulphate. The crystalline precipitate of albumin, precipitated by the addition of this salt and acidification to pH 4·6, was washed with an acidified 75 per cent saturated ammonium sulphate solution; this was followed by purification by dialysis in Cellophane sacs in running water and electrodialysis at 240 V against distilled water. The resultant transparent colourless solution of albumin of concentration 6 per cent had a pH value of 4·8 and gave no reaction for SO_4^{2-}; it was stored at 3° under toluene. From it a 3 per cent working solution at pH 8·0 was prepared before each experiment (4 ml of the original solution +3 ml H_2O+1 ml 0·1 N NaOH). Thanks to this alkalization the solution remained transparent throughout the most prolonged periods of irradiation.

The irradiation was carried out by a quartz-mercury lamp of low voltage; the two beakers each containing 4 ml of the working solution were placed at a distance of 9 cm below its tube. After irradiation the solution was placed for 1·5 hr in a thermostat at 40° for the completion of the "dark" stage of the photodenaturation, and brought to pH 4·8 by the addition of 0·1 N HCl. The precipitate of denatured protein filtered off easily. To the filtrate pure powdered ammonium sulphate was added in the proportion of 1 g salt to 1 ml filtrate. In order to hasten and facilitate the precipitation we tried keeping the protein solution containing the salt at 40°. It turned out, however, that even at this temperature in saturated $(NH_4)_2SO_4$ the albumin became denatured. Three hours' standing with the salt at room temperature was quite sufficient for complete precipitation; under these conditions the protein retained its native properties. The liquid was then filtered off; the precipitate was washed in the filter with 90 per cent saturated $(NH_4)_2SO_4$. The course of the washing away of the photo-products can be followed by measuring the ultra-violet absorption of the filtrate at $\lambda = 313\mu$ (at which the products of photo-oxidation absorb, but not the protein). We give an example of the diminution of the degree of absorption by

three fractions of washings of different volumes—the numbers are the extinction values of a layer of solution 6·3 mm thick; filtrate from the precipitation, 0·052; first washing, 0·034; second washing, 0·021; third washing, 0·017. These figures showed that there was no advantage to be gained by prolonging the washing.

After washing the precipitate was dissolved in the smallest quantity of water and was dialysed in a Cellophane sac against running water for not less than 40 hr under toluene, the inner level of liquid being kept higher than the outer in order to avoid dilution. The liquid was then poured into the middle section of a small three-chambered electrodialyser; in the side chambers, of capacity 15 ml, the cooled distilled water was changed periodically. The electrodialysis began at 160 V, the current strength not exceeding 35 mA. The latter is essential since, with large current densities and considerable concentrations of ammonium sulphate in the middle chamber, ammonium persulphate might be formed. This is probably the reason for the lack of success in our first experiments, where the solution from the middle chamber after the beginning of the electrodialysis energetically liberated iodine from KI, and the purified protein was strongly oxidized. This did not occur again when the duration of dialysis was as mentioned and the strength of the electrodialysing current was regulated. For complete purification three changes of water were sufficient. At the end of the electrodialysis a precipitate was thrown down, the current strength fell to 2 mA at 240 V and the solution became quite transparent. The purification was terminated by filtration from the precipitate.

The concentration of protein in the purified solution was within the limits 0·5 to 1 per cent, i.e. 3–6 times less than before the purification. This diminution cannot be explained merely by dilution during dialysis for the volume of liquid in the sac increased no more than twice. Evidently a considerable part of the protein is lost in the form of precipitate during the electrodialysis. This precipitation is in itself of interest; the non-irradiated solution of albumin worked up in exactly the same way remains quite transparent on electrodialysis.

Apparently our irradiated protein while not precipitated at the isoelectric point is all the same considerably less stable than the native protein and is partially denatured by the action of ammonium sulphate at room temperature. The longer the duration of the irradiation the more protein came out of solution. Only a quantitative investigation could show whether we are here dealing with a special form of "damage" to the protein by the irradiation—a damage slighter than photodenaturation. In any case after the electrodialysis there remains an albumin which is similar to native albumin in its stability in solution.

If the protein is separated from such a purified solution by heat coagulation there remains in solution a substance absorbing in the ultra-violet. This may be only photoproducts, precipitatable by saturation with ammonium sulphate but not passing through Cellophane, i.e. the highest molecular of the so-called "photo albumose". In fact addition of saturated $(NH_4)_2SO_4$ to the filtrate concentrated 6–10 times gives a turbidity and a flaky precipitate, at the same time liquid remains transparent on boiling. Its spectral absorption curve shows a uniform absorption without maxima from 248 to 280μ with a gradual falling off from 280μ to the long-wave parts of the spectrum. The maximum characteristic of aromatic amino-acid groups, i.e. of the very constituents that are known to undergo photo-oxidation in the first place,

is practically absent from this curve. As will be seen later the presence of these substances does not impede the work on the purified protein fraction.

2. TITRATION OF THE SULPHYDRYL GROUPS OF THE IRRADIATED PROTEIN

We were interested in a possible photochemical change in the "native" part of the irradiated protein. The destruction by irradiation of histidine and tryptophane in the protein structure, and the increase of the content of free amino-groups is already known. The photo-oxidation of the sulphydryl groups of the protein is also known as one of the easiest photoreactions of individual groups; it has even been used as an index of the change. It was formerly established qualitatively for egg albumin with the help of the nitroprusside test: the SH-groups have completely disappeared after irradiation, being oxidized to the S-S form. Such reagents as potassium ferricyanide and mono-iodoacetic acid react only with the active SH-groups of the protein. With their aid the active SH-groups in irradiated albumin solutions have been discovered and estimated quantitatively not only in the protein itself but in its photoproducts [4, 5]. It is evident that the appearance of these groups in irradiated protein solutions may be produced by photodenaturation (SH-groups of the protein) and by the photolytic action of the radiation (SH-groups of the photoproducts).

Thus specific reagents for active SH-groups, sensitive to the relatively slight changes in the condition of the protein, and their use for investigating photo-oxidation may only complicate the problem. For this purpose the iodometric method is more suitable.

Iodine liberated from potassium iodide by the addition of KIO_3 in strongly acid solution quickly and quantitatively reacts with SH-groups, active as well as masked, of egg albumin, and serves as a specific reagent for these groups [6]; KIO_3 can, therefore, under these circumstances be used directly for their estimation. During the time of its reaction with the SH-groups iodine does not oxidize S-S or other groups of the protein; if, therefore, by the action of the ultra-violet an oxidation of SH- to S-S-groups takes place, then in a rapid reaction the irradiated protein must react with relatively less iodine; which means that in the titration less iodate will be required for the irradiated than for the non-irradiated protein.

The iodate titration is carried out as follows. To 2 ml of the protein solution to be titrated there are added 3 ml N HCl, then 2 ml 5 per cent KI solution and three drops of starch solution. Then 0·005 N KIO_3 solution is run in dropwise from a microburette to a weak permanent dark end point. After acidification by HCl the protein forms a white cloud against the background of which the iodine starch reaction gives not a blue coloration, as in transparent solutions, but a brownish-grey. The end point is not very sharp and in order to detect it with an accuracy of 0·02 ml some practice is needed. With weaker solutions of KIO_3 the change is still less sharp and they should not be used.

For an experiment a 3 per cent solution of egg albumin is prepared and irradiated as described above. The irradiated protein is diluted with water to exactly the initial concentration. A part of the solution is worked up and purified to obtain the non-denatured fraction as described in section 1. Then by the method described above there were successively, titrated with iodate (1) 2 ml of the non-irradiated 3 per cent solution of albumin (2) 2 ml of the same solution, but irradiated (3) 4 ml of the

"native" fraction of the irradiated solution. For the last-mentioned sample 6 ml 1 N HCl and 4 ml 5 per cent KI solution were used. The results of the titrations of the first two samples can be compared directly; for the third sample it is necessary to calculate the dilution of the solution in the course of purification. For this purpose the concentration of the purified solution was determined from the weight of the heat-coagulum dried at 105°, and the number of millilitres of KIO_3 obtained for the titration was multiplied by the degree of dilution of the solution as compared with the original 3 per cent. In the transparent irradiated solutions, as in the non-irradiated, before acidification there was no precipitate. On strong acidification a cloud appeared more rapidly in the irradiated solutions, but at the time of titration all the solutions were equally cloudy so that in all cases the titration was carried out in the presence of a precipitate, and the protein reacting with the iodine was in considerable part already denatured by the acid.

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Such comparative titrations were carried out on a series of irradiations of different durations. Two samples of each solution were always titrated in turn. The difference did not exceed 0·02 ml iodate.

For the irradiated unpurified solution with exposures above 105 min in all experiments a diminution of the amount of iodate used in titrating SH-groups was obtained. For 2 ml non-irradiated 3 per cent solution 0·68–0·70 ml 0·05 N iodate were required; for 2 ml of solution irradiated for 2 hr or longer 0·57–0·46 ml iodate were required—depending on the exposure. We have expressed this diminution of the amount of titratable SH-groups, being the proportion of SH-groups not further oxidizable by iodine, in percentages of the initial quantity of titratable groups. The same diminution was given in the titration of the non-denatured fraction; here, however, the whole operation of purification and dilution, involving calculation back to the concentration, greatly increased the error of the determinations. The purified irradiated solution was usually obtained at a four- to five-fold dilution; 4 ml of the solution containing half as much protein as 2 ml of the non-irradiated. Even here after 2 hr irradiation the titration required 0·30–0·32 ml iodate, and after 4 hr 0·23 ml. These results—also in the form of the proportion of non-titratable groups—are given in Table 1 and also in the figure (curve 1, black circles), together with the results for the whole irradiated solution (curve 1, white circles) by way of comparison.

We give the results of control experiments. In order to elucidate the question as to whether the content of SH-groups of the purified irradiated protein had been affected by the working up during its separation we submitted a solution of the non-irradiated protein to exactly the same process. After salting out, washing, dialysis and electrodialysis the solution was titrated with iodate. For 4 ml of this solution of concentration 0·52 per cent there were required 0·24 ml of 0·005 N iodate, or—calculated for 2 ml of a 3 per cent solution—0·70 ml iodate; for 2 ml unirradiated untreated solution 0·71 ml iodate were required. This shows that the quantity of titratable SH-groups is not diminished in the course of the purification.

It was also confirmed that a part of the iodine is not used up in the oxidation of those photoproducts that, as already mentioned, remain after purification and separation of the irradiated fraction of the protein. According to Wels and Jokisch SH-groups are contained in the photo-breakdown products of protein. Attempting to apply the ferricyanide method for the determination of active SH-groups of

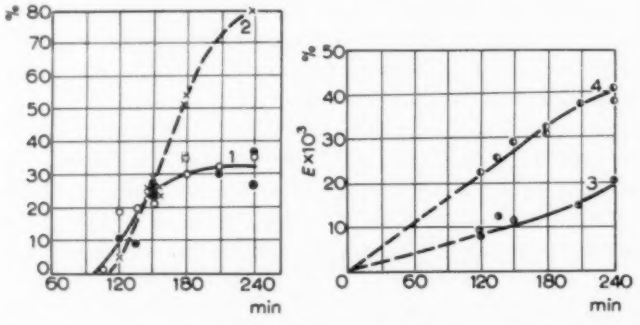
TABLE 1. PHOTO-OXIDATION of SH-GROUPS OF THE NON-DENATURED FRACTION OF IRRADIATED EGG ALBUMIN SOLUTION

Duration of irradiation (min)	SH-groups titrated (ml 0·005 N per 4 ml A) (a)	Relative concentration $\frac{A}{A_0}$ (b)	SH-groups titrated (ml 0·005 N KIO_3 per 2 ml 3 per cent solution A) (c)	SH-groups titrated A_0 (ml 0·005 N KIO_3 per 2 ml 3 per cent solution A_0) (d)	Proportion of non-titratable SH-groups A (per cent of SH-groups A_0) (e)
120	0·26	1 : 4·9	0·64	0·71	10
135	0·30	1 : 4·3	0·64	0·70	8·5
150	0·24	1 : 4·5	0·54	0·70	23
150	0·36	1 : 2·9	0·52	0·70	26
210	0·29	1 : 3·3	0·48	0·68	29·5
240	0·20	1 : 4·6	0·46	0·70	34
240	0·26	1 : 4·1	0·53	0·72	26

A_0 Non-irradiated original 3 per cent solution of egg albumin
A Solution of the non-denatured purified protein separated from the irradiated 3 per cent solution
a Average values of 2 or 3 titrations

$$c = \tfrac{1}{2} \times \frac{a}{b}$$

photo-denatured protein in an irradiated solution we ascertained that after 2 hr irradiation of a solution of egg albumin the photoproducts not linked with the protein reduce considerably more ferricyanide than could the whole protein from which they were formed. But, in titration with iodate of 2 ml of the solution of the purified, non-denatured fraction of irradiated albumin from which the protein had been removed by heat coagulation, a single drop (0·02 ml) of 0·005 N iodate gives a strong coloration. It follows that the photoproducts not combined with protein practically do not react with iodine. This was sufficient for checking the titration error.



Relation between quantity of oxidized SH-groups of egg albumin and time of irradiation, in comparison with other photochemical changes in the protein solution: (1) proportion of oxidized SH-groups in the irradiated solution (white circles) and in its non-denatured fraction (black circles), as percentages of the total SH-groups in the non-irradiated protein solution; (2) proportion of tyrosine groups liberated in the irradiated solution, as percentages of the total quantity liberated on complete denaturation; (3) quantity of photo-denatured protein as percentage of total protein; (4) absorption by the photoproducts in the irradiated solution for λ—3132 Å and a layer of 1·98 mm —$E \times 10^3$. Abscissae—time of irradiation in min

3. SUPPLEMENTARY DETERMINATION OF THE ULTRA-VIOLET ABSORPTION AND THE DENATURED PROTEIN CONTENT OF AN IRRADIATED SOLUTION

In order to compare our results on the protein SH-groups with the other previously described actions of radiation on proteins we have carried out determinations on the irradiated solutions of:

(1) the state of the tyrosine groups;
(2) the ultra-violet absorption of the photoproducts;
(3) the denatured protein.

The first of these determinations we have already reported in detail [2].

Here we will simply recall that part of the tyrosine-groups of egg albumin under the action of radiation or denaturation take on the property of free tyrosine of ionizing at pH 12, and at the same time the absorption in the region of 3,000 Å increases. The increase of the difference of degree of absorption between pH 12 and pH 9·5 is proportional to the proportion of changed groups. If the quantity of these groups liberated by complete thermodenaturation is taken as 100 per cent, then in any irradiated albumin solution it is possible from the difference of absorption to find the percentage of liberated groups by comparing this solution with a partially denatured solution of corresponding optical property. In doing this a correction is introduced for the optical properties of the photoproducts, which also give an increased difference of absorption at pH 12 and pH 9·5. The correction is calculated from the magnitude of this difference determined on a pure solution of the photoproducts and their relative concentration in the protein solution under investigation. All the necessary absorption measurements for the line $\lambda = 2967$ Å of the mercury lamp were carried out by the method previously described [1] at pH 12 and 9·5 for some of our solutions irradiated from 2–4 hr and the relative quantity of free tyrosine groups was determined. These results are given in curve 2 of the figure.

The absorption of the mercury line $\lambda = 313\text{m}\mu$ was also determined for a series of irradiated solutions. As was shown earlier [1] this magnitude is directly proportional to the total concentration of photoproducts and depends on their presence—the line $\lambda = 313\text{m}\mu$ is, in practice, but little absorbed by protein. The absorption of the photoproducts is calculated in the form:

$$\log \left(\frac{I_0}{I}\right)_1 - \log \left(\frac{I_0}{I}\right)_0$$

where $\left(\frac{I_0}{I}\right)_1$ is the ratio of the intensities of light falling on and transmitted by a layer of irradiated solution of definite thickness and $\left(\frac{I_0}{I}\right)_0$ the same ratio for the non-irradiated solution. A series of these values for solutions irradiated for 2–4 hr shows an increase of total concentration of photoproducts with increasing time of irradiation, and, consequently, the general course of photolysis is also given in curve 4 of the figure.

Finally, for some irradiated solutions the concentration of photodenatured protein was determined. For this a known volume of the irradiated solution was heated to 40°, brought to the isoelectric point and then filtered through a weighed filter. The filtrate was used for the preparation of the native fraction and the precipitate was

dried on the filter at 105° and weighed, and the concentration of the albumin precipitated at pH 4·8 in mg/ml was calculated. Some of the results obtained are represented by dots in curve 3 of the figure and mark out, though roughly, the course of the photodenaturation.

4. DISCUSSION OF RESULTS

The diminution of the content of SH-groups oxidizable by iodine in protein denatured by irradiation is set forth in the table and in curve 1 of the figure. There are no grounds for supposing any special blocking reaction under the action of ultra-violet of these groups easily accessible to iodine; it has already been shown that they are photo-oxidized to the S-S form. Photo-oxidized SH-groups cannot react with iodine during iodate titration. We therefore believe that the titration expresses the result of just such a photo-oxidation of the SH-groups of the albumin; from the table it is clear that this occurs even in the denatured fraction.

The fact that even before photodenaturation a marked photochemical change can occur in the protein (oxidation at approximately a third of all the SH-groups) is in good agreement with our earlier conclusions on the independence of photodenaturation and photo-oxidation, and that this latter reaction "outstrips" the photodenaturation [2]. As a considerable fraction—34 per cent—of the SH-groups of native egg albumin undergoes photo-oxidation the effect of ultra-violet irradiation in the presence of oxygen may be compared to that of a strong oxidizing agent.

As already mentioned the accuracy of the titration figures in the case of the "native" fraction is not great, and in themselves they are only a qualitative indication of the occurrence of photo-oxidation in this fraction. The error here amounts to 20 per cent and it is impossible to draw reliable conclusions as to the course of the reaction with time. However, it turned out that the values obtained were sufficiently close to the magnitude of the fraction of oxidized groups in the whole irradiated solution: the accuracy of these values was limited by the usual titration error (0·02 ml) with an error of the order of 10 per cent. In the figure, therefore, both sets of results are plotted: it is seen that they can both be referred to one common curve. This good coincidence is not surprising, since in the irradiated unpurified solution the non-denatured protein preponderates. With the exception of the 4 hr period of irradiation, when there is 20 per cent of denatured protein in the solution, in all the remaining cases its amount does not exceed 12 per cent. The quantity of photolysis products at such a degree of photodenaturation (according to the results of other authors and ourselves) does not exceed a fraction of a percentage of the total weight of protein; consequently their SH-groups can hardly affect the titration of the irradiated solution. This is not to say that the photoproducts do not contain a relatively high proportion of *active* SH-groups. This good correspondence of the results for both solutions allows us to regard curve 1 of the figure as correctly representing the course of photo-oxidation of the SH-groups of the non-denatured protein also.

We will call attention to some peculiarities of the course of the reaction. Only about one-third of the SH-groups enters into the reaction and the oxidation in practice goes no further than this. The slowing after 3 hr of irradiation is not caused by an increase of absorption of the radiation by the photoproducts: to show this we give curves 3 and 4 of the course of photodenaturation and the accumulation of photoproducts. Even from the few points obtained for these curves it can be seen that both

denaturation and general photo-oxidation still go forward very well at 3 and 4 hr irradiation.

Consequently the susceptibility of the SH-groups of egg albumin to the oxidizing action of ultra-violet radiation is not uniform; in 3 per cent solution at pH 8 and in the initial stage of the reaction about two-thirds of all the SH-groups are protected from oxidation. It should be emphasized, firstly, that the quantity of "photo-sensitive" SH-groups depends very largely on the concentration and pH of the solution (in our preliminary experiments) and, secondly, that on more prolonged irradiation a further more deep-seated stage of photo-oxidation may begin which takes in also the remaining SH-groups. The existence of this second stage of total photo-oxidation was shown by the results of our previous work [2].

One of the reasons for the rise of photosensitivity of the protein during the course of prolonged irradiation may be the denaturation. It is known how much the reactivity of protein SH-groups towards a series of oxidizing agents (cystine, ferricyanide, porphyrindine) increases on denaturation. The oxidant with which we are dealing seems to be no exception in this connection. This was shown by our experiment with thermodenatured protein.

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The non-irradiated 3 per cent solution of albumin at pH 8·0 with which the work has been carried out was denatured by 15 min heating in water at 85°. The solution, which had remained quite transparent, was then irradiated as described for 4 hr. After this it was titrated with iodate: 2 ml of the non-irradiated solution, 2 ml of the non-irradiated heat-denatured solution, and, finally, 2 ml of the heat-denatured irradiated solution were then titrated with iodate. The slowness of the reaction of the heat-denatured protein with the iodine liberated should be mentioned. A marked brown coloration of the whole solution is caused by the first drop of KIO_3; it disappears in 8–10 sec. The subsequent drops give a longer-lasting coloration: the end of the titration can be taken as a coloration lasting for 2 min. This relative slowness of reaction (iodine reacts practically instantaneously with the native protein) diminishes the accuracy of the titration.

The titration results in the above-mentioned order were: (1) 0·60 ml KIO_3; (2) 0·40 ml KIO_3; and (3) 0·12, 0·12, 0·16 ml KIO_3.

From this it is first of all evident that after heat-denaturation a part of the SH-groups does not react with iodine. Possibly this, and also the slowness of the reaction, is bound up with the oxidation of the SH-groups, or with an aggregation of the protein particles taking place in the still transparent solution, by the formation of intermolecular bonds the viscosity of our solution increases sixfold on heat-denaturation. However, of the titratable SH-groups of the denatured protein 67 per cent become oxidizable after 4 hr irradiation; consequently the photosensitivity of the SH-groups of this protein that are not concerned in intermolecular bonds is raised in comparison with the native protein—with respect not only to speed of reaction, but also to the number of accessible groups.

If this increase in the accessibility of the SH-groups takes place as the result of a relatively feeble action on the protein on denaturation, the possibility must be reckoned with of a much deeper photodecomposition, in which, with considerably more prolonged or more intense irradiation, probably quite different results would be obtained. Our results apply only to the initial stage of photo-oxidation of the protein.

The second peculiarity of the course of the reaction under investigation is the initial induction period.

The reaction virtually begins only after 105 min of irradiation. At about this time the "liberation" of the tyrosine groups begins (at low concentrations it occurs without any preliminary delay).

Curves 3 and 4 of the figure are of a different type; they pass through the origin. These reactions, photodenaturation and general photo-oxidation, proceed from the start of the irradiation with the final velocity. For the first of them, curve 3, there is a marked acceleration coinciding with the end of the liberation of tyrosine groups. This peculiar induction period is always observed in photodenaturation; we have already explained this by an increase of velocity of this reaction in photo-oxidized protein.

The presence of an induction period in the photo-oxidation of individual protein groups indicates the existence of several steps in the process.

We have already called attention in our previous work to the circumstance that the liberation of tyrosine groups goes forward almost to its conclusion with constant velocity. Under conditions of incomplete absorption of the radiation by the solution this would be explained if the speed of this "intramolecular" reaction were determined by some initial, much slower, process spreading in the form of a "front", or rather of two meeting fronts, penetrating the whole particle along one axis, and spreading along the chief chains.

Such a process gradually involving the protein particle and leading to the liberation of the tyrosine groups and the oxidation of the SH-groups might be the first stage of the photo-oxidation of the protein in the form of penetration of the oxygen dissolved in the water into the protein structure, and its photochemical combination with the protein—the photo-oxidation of the protein. It is known that photo-oxidation of the protein involves absorption of O_2.

The close correspondence of the beginning of curves 1 and 2 may indicate that the suggested photochemical union of the oxygen, which begins after 2 hr irradiation, serves as the initial step of the reactions of photo-oxidation of SH-groups and "liberation" of tyrosine groups.

The period of induction of these reactions shows that in a 3 per cent solution the beginning of this first stage of photo-oxidation in its turn requires some preliminary change that occurs with the participation of the radiation. This first stage of the photo-oxidation may be a change of the colloidal condition of the protein particles in solution their disaggregation. The second stage is the subsequent formation of an unstable oxygen compound with the protein structure. The last step in the reaction (oxidation of the SH-groups) could go forward in the presence of the oxidizing agent as a dark process, without participation of extra photons.

This concept of the initial stages of the photo-oxidation of protein is one of the possible explanations of the induction period observed in the photo-oxidation reaction. It still lacks experimental evidence.

CONCLUSIONS

(1) From an ultra-violet-irradiated solution of egg albumin an easily soluble fraction can be separated that is still capable of denaturation; it can be purified to a considerable extent from the products of photolysis.

(2) Such a protein possesses a partly reduced stability in solution, as compared with non-radiated albumin—it is partially denatured in saturated ammonium sulphate solution in the cold.

(3) A considerable fraction of the sulphydryl groups of the protein is oxidized and do not react with iodine: consequently the photo-oxidation of the SH-groups of the protein outstrips the photodenaturation and proceeds independently of it.

(4) On irradiating a 3 per cent solution of albumin at pH 8 the photo-oxidation amounts to 30–34 per cent of all the SH-groups of the native protein attains that react with iodine. With the photo-oxidation of these the reaction virtually comes to an end; consequently the remaining SH-groups in such a solution are protected against the action of radiation.

(5) On irradiation of a heat-denatured solution of egg albumin of the same concentration and pH the photo-oxidation reaches more than 65 per cent of all the SH-groups reacting with iodine.

(6) The photo-oxidation of the protein SH-groups shows an induction period; this may be connected with an initial change caused by radiation in the condition of the dissolved protein (disaggregation, for example), which precedes its photo-oxidation, or with the photo-oxidation of other more photosensitive groups.

Translated by T. R. PARSONS

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ACTION OF ULTRASONICS ON CARBOHYDRATES

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(*Received* 10 *June* 1956)

ULTRASONIC waves produce various chemical changes in bioactive materials. The rates and types of these processes depend on the structures of the compounds, on the solvent and its reactions and on the impurities it contains [1–4]. The ultrasonic intensity is also very important. The mechanism of the chemical (and also the biological) effect remains quite unclear, however; this topic is the subject of fairly lively discussion.

There is no doubt that the chemical processes occur when the wave intensity is sufficient to initiate cavity production. There are reasons for thinking that the chemical

processes are due to there being the products from splitting ionized water molecules in the solution: these latter being highly reactive:

$$H_2O - e \rightarrow H_2O^+ \rightarrow H^+ + OH;\ H_2O + e \rightarrow H_2O^- \rightarrow OH^- + H.$$

The water is ionized during cavitation, high electric fields being produced in the cavities [5, 6]. The decomposition products from ionized water molecules pass into the surrounding water when the bubbles collapse as the waves move on: they then react with solutes.

A number of effects observed with ultrasonics can be explained on this basis, particularly those shown with the carbohydrate solutions we studied. There is a definite regularity in the sequence of chemical changes caused in these compounds by ultrasonics.

In addition, it was possible to show that there were some groups generally present in the final products from all the sugars irradiated, as well as from some related compounds.

Szent-Györgyi [7] first observed the chemical action of ultrasonics on carbohydrates in 1933. Dextrin-like substances were produced by irradiating starch solutions. Gohr and Wedekind [8] found that irradiating a 1 per cent glycogen solution for many hours produced reducing substances. According to Khenokh [9], reducing agents are also found in solutions of mono- and di-saccharides under these conditions.

The break-up of starch, glycogen and mono- and di-saccharides was only found after prolonged (10–15 hr) irradiation of the solutions in these cases; this being due, in the author's opinion, to oxidation by hydrogen peroxide and nitric acid. When oxygenated water was irradiated for long periods it was in fact found that amounts of hydrogen peroxide and nitric acid detectable by ordinary methods accumulated [10].

But, as will be shown, under some conditions the carbohydrates show definite chemical changes after much shorter irradiation (order of minutes), which implies a distinct mechanism for the chemical (and biological) action of ultrasonics, more specific for this physical agent.

EXPERIMENTAL PART

Ultrasonic effects were studied in mono-, di- and tri-saccharides: arabinose, glucose, saccharose, maltose and raffinose. All irradiations were carried out at the same frequency (385 kc/s) and intensity (4–5 watts/cm² of radiator). The emitter was a plane-parallel quartz plate of diameter 5 cm. The crystal-holder allowed the ultrasonics to enter the water directly. To avoid breakdown at the ends of the plate it was immersed in transformer oil: there was an air layer under the lower surface of the plate.

Irradiations were performed in glass test-tubes containing 10 ml of solution. The tubes were placed in water, whieh served as the contact liquid between tube and quartz plate.

The plate-tube distance did not alter. Only the duration of irradiation was altered. The solution temperature did not exceed 25–30°C. The solution was kept cool by being in contact with the water transfer medium, itself cooled by a cooling coil.

All materials were studied by ultra-violet spectrophotometry before and after irradiation (SF-4 spectrophotometer).

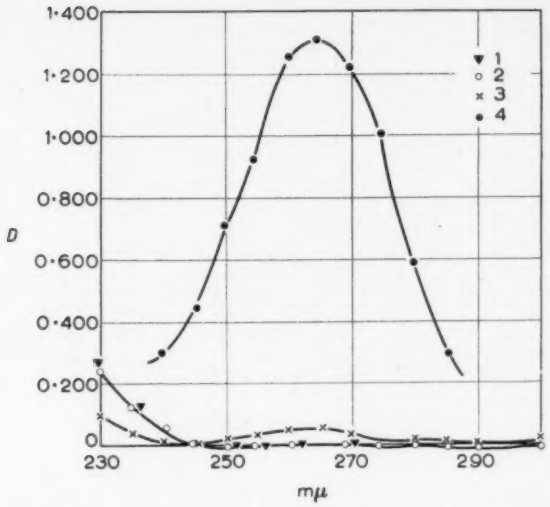


FIG. 1. Absorption curve of an irradiated glucose solution ($3{\cdot}3 \times 10^{-3}$M). D, optical density. Irradiation time, 15 min at following pH values: 1—pH 7; 2—pH 9; 3—pH 11; 4—pH 13.

The amounts of reducing substances in irradiated and non-irradiated solutions were also determined: pH values were taken with a glass electrode.

All the sugars used gave rise to compounds having absorption maxima at 265 mμ when irradiated in aqueous solution. This was already apparent with short irradiations (5–10 min), but was strongly dependent on the pH. It was only found when the pH was about 13 (Fig. 1).

All the sugars were subsequently dissolved in 0·1 N NaOH.

Figs. 2 and 3 show that the absorption at 265 mμ rises with the irradiation time. There is a linear relation between the amount formed, as judged from the absorption maxima, and irradiation time, according to our data. The rate of formation of absorbing material begins to drop when the sugars are irradiated for very long periods.

Our data (which agree with those of Gabryelski and Marschlevski [11]) show that certain sugars (glucose, arabinose and maltose) split up into various substances on heating in 0·1 N NaOH solution on a boiling water bath for 20–30 min: some of these have ultraviolet absorption maxima at 265 mμ (Fig. 4). These sugars usually give a yellow colour when so heated in alkaline solution, whereas they remain colourless and transparent after ultrasonic irradiation.

The alkali solutions of the other sugars used (saccharose, raffinose) give neither visual nor spectroscopic changes on heating on a boiling water bath for longer periods (1–2 hr). The spectra show that these also undergo the same changes as glucose, arabinose and maltose on ultrasonic irradiation (Fig. 5).

It is clear that the first group (glucose, arabinose and maltose) comprises sugars with free aldehyde groups at the ends of the molecules, while those in the second group (saccharose and raffinose) incorporate the same sugars, but with aldehyde groups blocked.

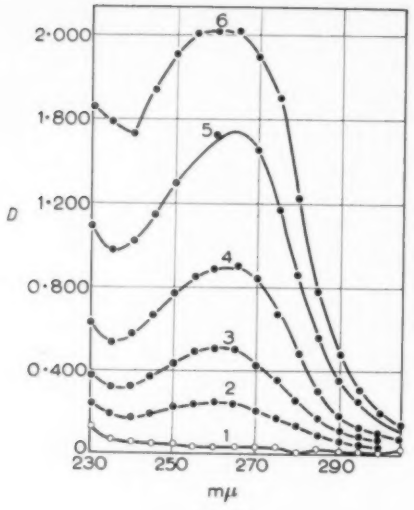


FIG. 2

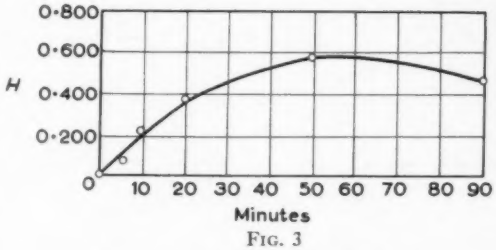
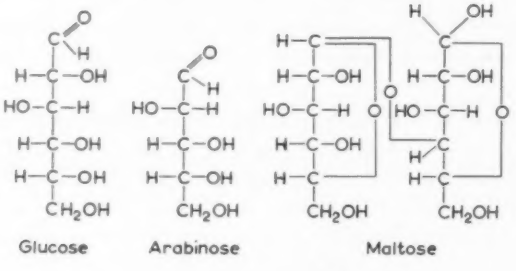


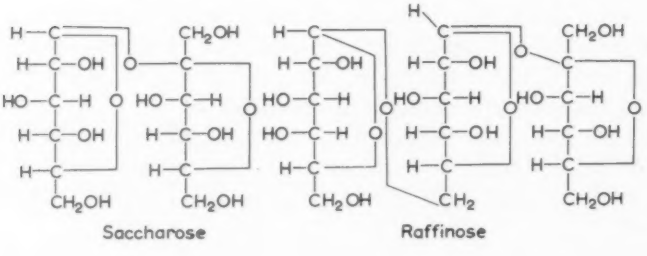
FIG. 3

FIG. 2. Absorption curves of an arabinose solution (4×10^{-3}M) in 0·1 N NaOH, before and after irradiation; D, optical density. 1, before irradiation; 2, irradiation for 5 min; 3, 10 min; 4, 20 min; 5, 50 min; 6, 90 min.

FIG. 3. Absorption maximum height, H, for an arabinose solution (4×10^{-3}M) as a function of irradiation time.

Enolization occurs in alkaline media when there is a free aldehyde group in the sugar molecule, this rendering the molecules more reactive, according to Kuzin [12]. This clearly explains why glucose, arabinose and maltose undergo extensive and varied decomposition in alkaline media at high temperatures. The decomposition products, as has been stated, include those which give ultra-violet absorption maxima at 265 mμ.





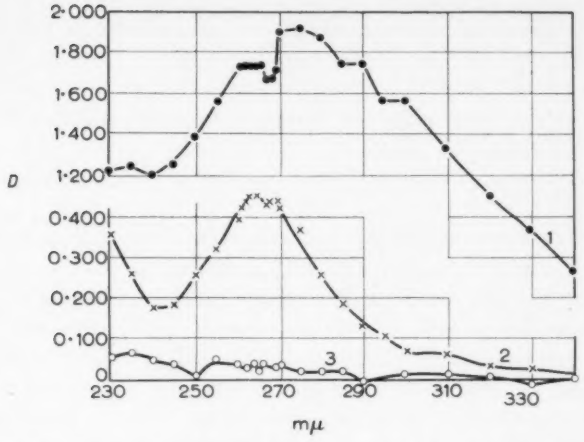


FIG. 4. Absorption curve for a maltose solution ($1{\cdot}6 \times 10^{-3}$M) in 0·1 N NaOH. 1, absorption spectrum (D, optical density) of a maltose solution after heating for 10 min on a boiling water bath; 2, absorption spectrum of a maltose solution irradiated for 10 min; 3, 2 before irradiation.

Somewhat different changes due to ultrasonics are found with group 2 sugars. This is shown by the solutions staying colourless. But materials with absorption maxima in the ultra-violet region occur in them.

Hence it was supposed that saccharose and raffinose undergo no thermal changes because the aldehyde groups are blocked. The aldehyde groups are only liberated by rupture of the oxygen bridges between the mono-saccharides, produced by

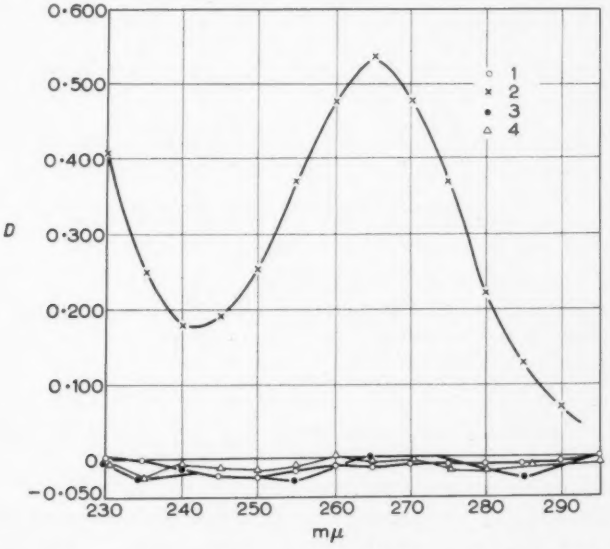


FIG. 5. Absorption curve of a raffinose solution (10^{-2}M) in 0·1 N NaOH. 1, before irradiation; 2, irradiated for 10 min; 3, heated for 30 min; 4, irradiated with traces of ethyl ether.

ultrasonic action, these groups appearing in the medium in the enol form, and thus facilitating further changes in the irradiated sugars.

The liberation of aldehyde groups in saccharose and raffinose solutions by ultrasonics is shown by the occurrence of reducing substances which increase in amount with the irradiation time (see Table).

The Hagedorn-Jensen micro-method was used to determine the reducing substances.

The tabulated data show that the reducing substances are formed by the ultrasonics in both acid and alkaline media. If a saccharose solution, irradiated while acid, is brought to pH 13 by adding alkali, subsequent heating causes further decomposition, giving products with absorption maxima at 265 mμ in the ultra-violet. Saccharose does not give this even on prolonged heating in alkali if not first irradiated, as has been stated. Ultrasonics also give the reducing substances when applied to solutions of complex alcohols, such as mannite (see Table). Further, mannite, like saccharose, glucose and the other sugars, gives an absorption maxima at 265 mμ when irradiated in alkaline solution (but not on heating). (Fig. 6)

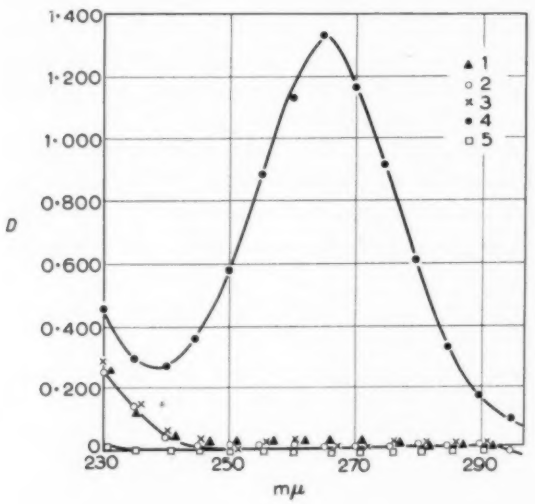


FIG. 6. Absorption curves for mannite ($3{\cdot}3 \times 10^{-3}$M) at various pH values. Irradiation time, 10 min, at various pH values: . . . with traces of ethyl ether.

If the previously irradiated acid mannite solution is brought to pH 13, heating causes a further break-up, producing substances with the same type of absorption band (Fig. 6).

Similar results were obtained on irradiating glycerol (Fig. 7 and Table).

Complex alcohols soluble in water may be supposed to be oxidized by ultrasonic irradiation, giving aldehydes which enolize in the alkali. The latter enolization facilitates their further change on irradiation or heating, as was shown by spectrophotometry.

TABLE 1. AMOUNTS OF REDUCING SUBSTANCES IN IRRADIATED SOLUTIONS OF SACCHAROSE, RAFFINOSE AND OTHER ORGANIC COMPOUNDS

Compound	Concentration (M)	pH value	Amount of reducing substance (in mg of glucose per ml) on irradiation for			
			0 min	10 min	30 min	60 min
Saccharose	$1{\cdot}7\times10^{-3}$	13·0	0·003	0·038	0·070	0·105
Saccharose	$1{\cdot}7\times10^{-3}$	3·9	0·007	—	0·058	—
Raffinose	$1{\cdot}0\times10^{-3}$	13·0	0·017	0·042	0·056	0·083
Mannite	$3{\cdot}3\times10^{-3}$	13·0	0·009	0·040	0·062	0·087
Mannite	$3{\cdot}3\times10^{-3}$	3·9	0·009	—	0·058	—
Glycerine	0·68	13·0	0·006	—	0·076	0·151

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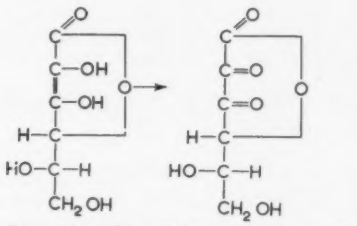
The final products from the sugars and complex alcohols were detected spectrophotometrically by adding hydrochloric acid to pH 2 to the irradiated alkaline solution. This shifts the maxima towards the short-wave end, i.e. to 245 mμ instead of 265 mμ. Organic compounds with conjugated double bonds give absorption maxima in this region of the ultra-violet [13].



Ascorbic acid is one such compound.

Like the sugars and complex alcohols, ascorbic acid gives an ultra-violet absorption band, the maximum varying in position with the pH, being at 265 mμ in acid, and 245 mμ in alkali. The height of the maximum falls continuously on irradiation, which implies that the compound is gradually destroyed during irradiation. We should therefore also assume that the compounds with double bonds formed by irradiating the sugars and complex alcohols also decompose in part. This effect probably alters the yields of the reactions.

The above chemical effects do not as a rule occur (see Figs. 5 and 6) when small amounts of ethyl ether (0·1 ml) are present in the solutions we used.

Ethyl ether, in common with other substances having vapour pressures higher than that of water, evaporates in the cavitation bubbles, and retards the ionization of the water, thus preventing free radicals from being formed—these being the decomposition products from the solutes.

The chemical reactions induced by the ultrasonics are in all probability caused by electrochemical processes occurring in the cavitation voids.

The data given here must be taken into account when analysing the mechanism

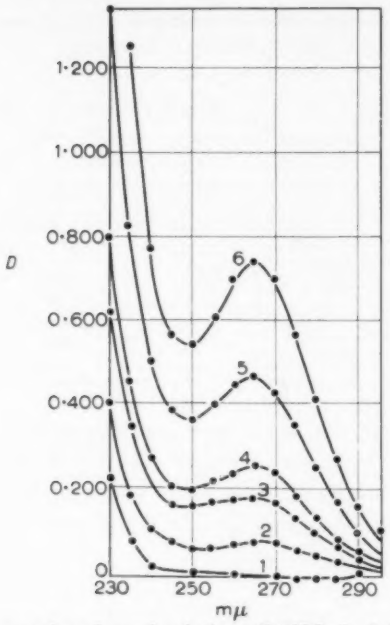


FIG. 7. Absorption curves for glycerol solutions (0·68M) in 0·1 N NaOH. 1, before irradiation; 2, irradiated for 20 min; 3, 40 min; 4, 60 min; 5, 120 min; 6, 180 min.

of ultrasonic biological effects, particularly when the reactions in the living organism are due to cavitation within or without it.

CONCLUSIONS

(1) Glucose, arabinose and maltose, which have free aldehyde groups, give substances with ultra-violet absorption maxima at 265 mμ when irradiated with ultrasonics in alkaline media and also on heating.

(2) Alkaline solutions of saccharose and raffinose also give absorption maxima at 265 mμ if previously irradiated, but do not do so on heating. These organic compounds give substances with reducing properties when irradiated in both acid and alkaline media.

(3) Irradiated alkaline and acid solutions of complex alcohols and glycerol also show reducing properties. In alkaline solutions they give substances with absorption maxima at 265 mμ.

(4) The prior irradiation of acid solutions of saccharose, raffinose and mannite, subsequently brought to pH 13 with alkali, leads to just the same changes on heating as are found with glucose, arabinose and maltose.

(5) Discussion of the experimental data leads to the following conclusions:

(*a*) Ultrasonics break the oxygen bridges in saccharose and raffinose, freeing aldehyde groups. Complex alcohols are oxidized under these conditions, giving the same group. The processes are not pH–dependent.

(*b*) Further changes occur both on ultrasonic irradiation and on heating if the operations are carried out in alkaline solution, in which the enol form of the aldehyde group predominates.

Translated by J. E. S. BRADLEY

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ON THE INFLUENCE OF ILLUMINATION ON THE ELABORATION OF COMPLEX AUDITORY REACTIONS*

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Night is one of the most powerful factors in the environment and, without doubt, exerts a great influence on the human organism—mainly on the nervous system, where it raises or lowers the excitability of the cerebral cortex. And this may substantially affect the activity not only of the human visual analysor, but also of other analysors, among them the motor analysor, and the rate and accuracy of movements, and the rate and degree of elaboration of complex motor reactions.

These questions, however, have been hardly investigated up to the present, although their study would on the one hand elucidate certain inter-relationships of the visual and motor analysors and, on the other hand, would allow of a more exact choice of the optimal conditions of illumination, both for the elaboration of various work-habits, and for their utilization under working conditions. The object of the present work is to establish precisely the influence of illumination on the elaboration of work-habits in workers in construction trades (crane operators, excavator drivers).

For the elucidation of these questions, we set up in the laboratory such experimental conditions as reproduce those of the work of crane operators or excavators, and decided to follow in the subjects the refining of visual analysis, and the elaboration of motor-habits in relation to the conditions of illumination.

EXPERIMENTAL TECHNIQUE

The experiments were carried out with the equipment described by Kanavets-Iakovleva [1].

* Paper presented to the Fourteenth All-Union Congress of Physiological Optics, Leningrad, October 1955.

At a distance of 4·75 m from the subject a vertical dark-mat metallic rod was set up (coefficient of brightness = 0·15). Its thickness subtended an angle of 2′ at the observer. Above this rod, there was suspended, on three ropes leading to the subject's place, an electromagnet (load), holding an exactly similar metal rod. Working with two hands and a left pedal, the subject could shift the upper rod, within wide limits, around the lower rod.

The task of the subject was to place the hanging rod so that it occupied a position exactly above the lower rod, but not touching it, i.e. the subject had to bring the two rods vertically into line.

In part of the experiments the rods were moved by the experimenter, the subject stating the moment at which they coincided.

After placing the two rods into line, the subject pressed a right pedal by which the circuit of the electromagnet was broken, and the upper rod fell either on to the lower rod or on to a scale below, by which the experimenter judged the degree of accuracy of the placing.

In order to exclude possible additional orientations in the subject's field of vision, there was placed in front of the rod an opaque screen with a rectangular aperture (80 × 30 cm), through which the subject could see only the background, the electromagnet with the suspended rod, and 3–4 cm of the lower rod above the lower edge of the aperture.

The average value of brightness of the background and the screen was 0·3. The illumination of the background screen and rods was uniform. It could be changed with the help of metallic blinds in the lamps, and partly by means of a rheostat.

All the movements of the two handles and pedals were recorded, so that we could judge of the nature, number and duration of the movements and pauses, for each hand and leg separately.

Before each experiment, the subject was adapted, for 20 min, to the illumination maintained in the experiment. The position of the subject's head was not fixed in our experiments.

At the beginning of the experiments, we did not explain to the subjects that we wished to obtain from them results with the minimal time and number of movements with a maximum accuracy of placing. Only after 2-3 experiments, when the subjects began to work with more certainty, were they requested to place the rods more quickly without diminishing the accuracy. They were not informed of the accuracy with which they placed the rods.

Before each setting the subject had to release both pedals and, with the help of the handles, to bring the upper rod to an extreme right or left position, indicated arbitrarily by the experimenter, then at once to begin the setting. If the setting always began from one starting point, the subjects could rapidly develop a stereotyped motor-habit. In all 106 experiments, with 18 subjects, aged 18–25 years, with normal colour perception and visual acuity, were carried out. All the experiments were made in 3 series with 6 subjects in each. In the first series, the subjects worked at an illumination of 300 lux, in the second series of 10, and in the third series of 1 lux. Such illuminations often occur in the artificial lighting of constructional sites.

EXPERIMENTAL RESULTS

(a) *Features of the elaboration of complex motor-reactions.* Let us consider the features of the elaboration of complex motor-reactions observed in all our experiments independently of the level of illumination.

Fig. 1 shows typical mechanograms for 3 placings, characteristic of the process of elaboration of complex motor-reactions.

The problem of placing the two rods in a vertical line required a complex coordination of the movements of both hands and the left leg, and, in the first experiments, proved to be very difficult for all the subjects. For the achievement of the first settings, individual subjects required from 1 to 5 min. As is seen from Fig. 1(a), the subjects performed many "hunting" movements and often, particularly in the beginning of the elaboration, tried to work either with one hand or foot alone in turn, and only later began to work with both hands and the left leg (Fig. 1b), and to arrive at more fluent movements, often leading at once to the setting of the rods (Fig. 1c). The process of formation and reinforcement of complex motor-reactions in our experiments (Fig. 1) involved the shortening of the time of the settings, the gradual disappearance of excessive movements, and the diminution of the number and duration of the movements and pauses.

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It is worth mentioning that the relation of the duration of all the movements to the duration of all the pauses during the individual settings at various levels of illumination was the same in all the experiments. The movements of the hands occupied 20–25 per cent and the movements of the left foot 10–15 per cent of the total time of the settings. Even after the elaboration and reinforcement of the motor-reactions, when the subject placed the rod at once, without any extra movements, after the setting he made a pause of 3 times the duration of the movements.

In the analysis of the mechanogram after each setting, we calculated the total time of the settings, the number and duration of the movements and pauses of both hands and of the left foot, and the accuracy of placing of the rods. Movements of

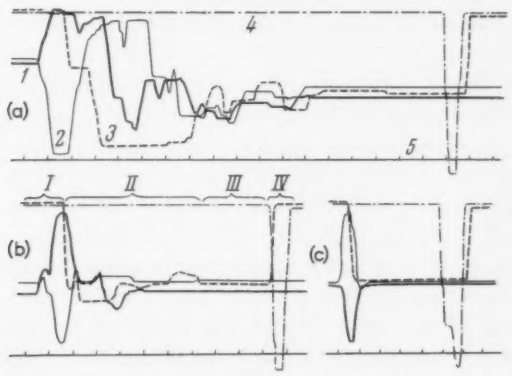

FIG. 1. Mechanogram of settings (subject E.Z.): (a) fiftieth, (b) sixty-fourth, (c) hundredth. Mechanograms of movements: (1) right hand; (2) left hand; (3) left foot; (4) right foot; (5) time signal (5 sec); (I) taking of load to initial position; (II) movements leading to setting of the rods; (III) pause after setting; (IV) pressing of pedals. The shortening of the total duration of the placing and the diminution of the number and duration of the movements and pauses during the elaboration of the complex motor reactions is seen.

the right leg, denoting the completion of the setting, were recorded but not taken into account, because they were the same in all the experiments.

Total duration of the settings, the extent and duration of the movements and pauses for each hand and the left foot, and the magnitude of the error in the placing, varied in all the subjects from setting to setting. It was, therefore, found to be expedient in the analysis of the basal results to take the average of the results of five successive placings of the rods by each subject.

Comparing the duration and extent of movements of the right and left hands, it is seen that at all levels of illumination the movements of the left hand over the range of all the experiments was, on an average, 1–2 times as great as the movements of the right hand. The duration of the movements of the left hand was also almost always somewhat greater than the duration of movements of the right hand. This can evidently be explained by the greater use of the right hand during the subjects' earlier life.

Seeing that the duration and extent of movements of the right and left hands show the same regularity, and that, in our experiments, the number and duration of the pauses had a constant relation to the number and duration of the movements, we shall in the following, not take into account the indications of the left hand or the number and duration of the pauses.

With each subject, we carried on the elaboration of the motor-reactions until there was no longer any reduction in the time of placing and in the extent of movements, and until there was established a certain movement-pattern. Using various levels of illumination, we found that the speed and degree of elaboration of complex motor-reactions depended to a considerable extent on the illumination.

(b) *Changes of rate and limits of the elaboration of complex motor-reactions in relation to illumination.* In all the subjects qualitatively simple changes in the process of elaboration of complex motor-reactions were observed; we therefore averaged the results of the experiments with all the subjects of each series in order to elucidate the general regularities.

In Fig. 2 the averaged results of the total duration, errors in the setting of the rods, duration and number of movements of the right hand and left foot for all three series of experiments are given. From Fig. 2 it is clearly seen that the lower the level of illumination the slower and more prolonged the elaboration of complex motor-reactions. With illumination of 300 lux the elaboration and reinforcement of the motor-reactions required 80 settings, with illumination of 10 lux. This required 130 settings, and with illumination of 1 lux, 250 settings. Even when the complex motor reactions had been elaborated and reinforced, subjects having worked at 1 lux showed worse records after 250 settings than the subjects who had worked at 10 and 300 lux.

At the lower levels of illumination the subjects showed many "hunting" movements, and made many long pauses; sometimes, after a series of movements not leading to the setting of the rods, the subjects made no movements at all for some minutes. The movements leading at once to the setting of the rods became reinforced extremely slowly. A lowering of the curves, in all the records, occurred markedly more slowly at 1 and 10 lux than at 300 lux.

On considering the general curves for all the experiments of the three series (Fig. 2), it can be seen that the total time of the settings and the duration of the movements of the right hand and left foot, at the beginning of the elaboration of

the complex motor-reactions, were least at an illumination of 300 lux, greater at an illumination of 10 lux, and still greater at an illumination of 1 lux. At the end of the elaboration the curves tended to approach one another. The curves of the movements of the right hand and the left foot were distributed somewhat differently. Least movements at 300 lux, somewhat more at 1 lux, and still more at 10 lux.

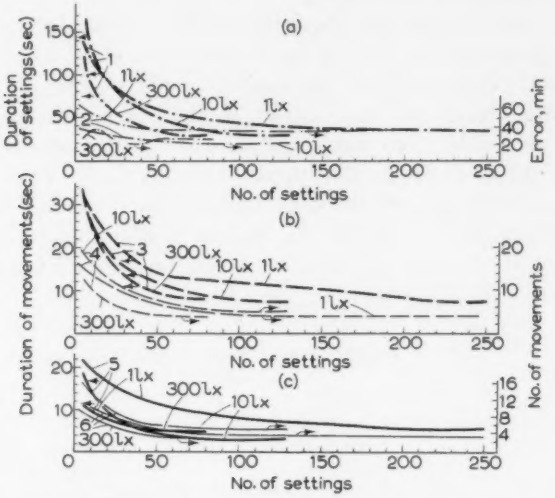

FIG. 2. Changes of total duration of placing, error, number and duration of movements of the right hand and left foot during elaboration of complex motor-reactions in relation to illumination:
a: (1) total duration of the settings, (2) error.
b: (3) duration of movements of right hand, (4) number of movements of right hand.
c: (5) duration of movements of left foot, (6) number of movements of left foot.

Thus, at high illumination, the total duration of the motor reactions, and also the number and duration of the individual movements, were less than at lower illuminations.

(c) *Relation of visual analysis to the motor-reactions.* It is known that the accuracy and speed of visual analysis depends, to a large extent, on the illumination of the object. At greater illumination visual analysis proceeds more quickly and more accurately. We judged the accuracy of the visual analysis by the magnitude of the error in the placing of the rods by the subject himself. In all three series of experiments, at the sixtieth placing, there became established a certain average level of error, which, subsequently, remained practically unchanged. Under these conditions of experiment, the error was highest at 1 lux. At the beginning it amounted to 60 mm, and later to 35 mm. At 10 lux the error at the beginning was 35 mm, and later 20 mm. At 300 lux, the errors at the beginning and at the end of the elaboration were both 25 mm. These results were obtained with the participation of the complex motor-reactions in the process of visual analysis of the object.

For the elucidation of the influence of the complex motor-reactions on visual perception, we carried out in the course of the basal experiments a series of confirmatory experiments in which the placing of the rods was carried out by the

experimenter, instead of the subject. When the subjects themselves placed the rods, they announced verbally the moment of coincidence of the rods.

Under these experimental conditions, the error in the overwhelming majority of the answers of the subjects was considerably less than in the placing of the rods by the subjects themselves (Table 1). It was only in individual cases that the average magnitude of the error was unchanged.

It was found that, in the cases where the subjects responded verbally, and not by complex motor-reactions, the speed of visual analysis was also considerably increased. While in the basal experiments all the subjects made a pause of about ½ min after the setting, during which time the accuracy of the placing was estimated, when the upper rod was moved by the experimenter, the subjects at once announced the moment of coincidence of the moving upper rod with the lower.

These facts indicate that, in the experiments, the speed and accuracy of visual analysis were influenced not only by the degree of illumination of the objects, but also by the nature of the response, whether verbal or a complex motor-reaction.

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TABLE 1

Subject	Average magnitude of error in perception of depth (mm)			
	In the first experiments		In subsequent experiments	
	Motor response	Verbal response	Motor response	Verbal response
P.L.	45	5	50	14
	36	14	27	30
M.S.	50	12	43	26
	30	2	42	1
A. Ia.	38	9	23	10
	66	8	35	35
I.M.	46	10	40	0
	52	10	77	12

DISCUSSION OF RESULTS

The facts obtained in our experiments indicate that the conditions of illumination exerted a considerable influence on the speed and extent of the elaboration of complex motor-reactions. In Table 2 are given the average values of the duration of the settings, the error, the duration and number of movements of both hands and left foot obtained at various illuminations, at the beginning of the elaboration of the complex motor-reactions, and at the eightieth setting, at which the elaboration of the reactions at 300 lux was terminated.

Analysis of table: (1) the total duration of the settings, both at the outset of the elaboration of the reactions, and particularly in the subsequent settings, was considerably greater at lower illumination. (2) The duration of movements of both hands was at the beginning approximately the same at all illuminations but the duration of the movements of the left foot, both at the outset of the elaboration and in the subsequent settings, was considerably greater at the lower illumination. (This is evidently connected with the fact that, under the conditions of our experiments, the

TABLE 2

	Illumination (lux)					
	300		10		1	
	At outset of elaboration of reaction	At eightieth setting	At outset of elaboration of reaction	At eightieth setting	At outset of elaboration of reaction	At eightieth setting
Total duration of settings (sec)	117	30	175	45	137	54
Duration of movements (sec)						
Right hand	34	7	31	10	32	13
Left hand	34	7	31	9	37	15
Left foot	11	4	19	4	22	7
No. of movements:						
Right hand	10	4	19	7	15	6
Left hand	11	4	20	8	17	7
Left foot	8	3	16	6	11	4
Magnitude of error (mm)	25	25	35	20	60	35

movements of the hands did not demand particular accuracy, since they led only to the coarse adjustment of the upper rod in line with the lower; the final placing of the rods was carried out by accurate movements of the left foot; the diminution of the illumination, therefore, told more on these motor-reactions.) (3) The numbers of movements of both hands and the left foot were also greater at lower illumination both at the outset of the elaboration and in subsequent settings. (4) The accuracy of perception of depth was less at lower than at higher illumination.

At the end of the elaboration of the reactions, all the values given have become approximately identical, but if the elaboration of complex motor-reactions required 80 settings at 300 lux, at 10 lux 130 were required, and at 1 lux 250 settings.

Thus, at lower illumination the number of repetitions necessary for the elaboration of the complex motor-reactions sharply increased. At the same number of repetitions the number and duration, particularly of the accurate movements, also increased. Together with this, there is a reduction of accuracy of the perception of depth.

For the analysis of the functional mechanism of the phenomena discovered, it is important to take into account that change of the conditions of illumination produces a different level of excitability of the visual analysor. By virtue of the phenomena of irradiation the excitability of other parts of the cerebral cortex, including the motor analysor, is also altered. Light of various intensity produces in the cerebral cortex different "backgrounds" of excitability on which the complex inter-relations between the thresholds of excitability of the motor and visual analysors develop. Excitation in the visual analysor by higher level of illumination irradiating in the cerebral cortex may lead to a rise of excitability of the motor analysor, and so determine a more rapid elaboration and reinforcement of the complex motor-reactions.

The difference of excitability of the cortical termination of the visual analysor, produced by different illumination, is shown by investigations of the electrical excitability of the visual analysor on the transition of the eye from light to dark and

vice versa. In these investigations, it was established that the electrical excitability of the cortical termination rises in the light and falls in the dark.

These facts have led the authors to suggest that in the process of dark adaptation the excitability of the cortical termination of the analysor is lowered [2–4].

Is the deterioration of the complex motor-reactions connected with a deterioration of visual analysis at lower illumination?

The results of our experiments showed that the limiting rise of accuracy of placing was attained at the sixtieth setting, while the motor reactions at analogous illumination developed at the eightieth, one-hundred and thirtieth, and two-hundred and fiftieth setting. This indicates that the rate of elaboration of the complex motor-reactions was not entirely determined by the change of visual perception, and that, at the basis of this fact, there lies, apparently, a different excitability of the motor analysor at different illumination.

Our experiments have shown that, with complex motor-responses, visual analysis is inferior to that with verbal answers reinforced by a previous live experiment. The motor-reactions themselves lead to a considerably greater deterioration of the visual analysis than did a reduction of illumination from 300 to 1 lux. Without the motor reactions, the speed and accuracy of depth perception were several times greater than in combination with motor-reactions.

It can be suggested that, at the basis of these phenomena, there lie complex induction inter-relationships arising in the cortical parts of the visual and motor analysors. The elaboration of complex simultaneous motor-reactions of both hands and left foot produces a centre of enhanced excitability. A wave of induced inhibition, spreading through the cerebral cortex, may invade the visual analysor, reducing therein the level of excitability determined by the light-energy perceived by the eye [5, 6]. The result of this is a reduction in accuracy and speed of visual analysis.

CONCLUSIONS

On the basis of the present work, the following conclusions can be drawn.

(1) The speed of elaboration of complex motor-reactions depends, to a considerable extent, on the illumination: with higher illumination the elaboration is accelerated, and with lower illumination is retarded.

(2) The total duration of the motor-reactions and the duration of movements at an equal number of repetitions is considerably greater at low illumination, particularly when the greatest accuracy of movement is required. At the end of the elaboration, these differences smoothed out.

(3) The number of movements necessary for the solution of one and the same problem, with an equal number of repetitions, is considerably greater at lower illumination.

(4) The accuracy of depth perception was higher with higher illumination at the time of the performance of the complex motor-reactions. It was many times greater in the absence of the motor reactions.

(5) The improvement of the activity of the motor-analysor, and the deterioration of visual analysis performed simultaneously with complex motor-reactions, must be taken into account in practice, in the provision for manual workers (particularly where speed and accuracy of movements are required) of such an illumination as will

give the maximum accuracy of visual analysis and efficiency of the motor analysor.

I express my thanks for advice and help to V. G. Samsonova and M. V. Sokolov.

Translated by T. R. PARSONS

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DISTURBANCE OF MUSCLE CO-ORDINATION IN HIGH MOUNTAIN CONDITIONS

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(*Received* 15 *October* 1955)

THE influence of altitude factors on movement co-ordination in man occupies an extremely modest place in the numerous investigations into physiological functions under high mountain conditions, in spite of the exceptional importance of these functions. The literature data on indices of co-ordination, such as reaction rates, are contradictory.

During and Reichel [1] admitted that an increased reaction speed was possible. Barcroft and Murray [2] found a reduced reaction speed and Bonnardel and Liberman [3] did not detect any change (all these at heights between 4,200 and 5,400 m). Later work did not define or clarify this problem. The data from single observations on more complicated voluntary movements are just as contradictory, this being due to the short duration of pressure-chamber tests and to the even shorter periods of flight without oxygen equipment [4, 5, 6, 7, etc.].

In none of these papers have we found concrete evidence of systematic investigation into co-ordination of movements under high altitude conditions of long duration, or perhaps on sufficiently prolonged pressure-chamber tests. Attempts at interpreting disco-ordination mechanisms, recorded in various circumstances, seem speculative in view of the insufficient factual material.

In short, not one of the investigations was primarily concerned with co-ordination of movement.

The activity of the motor analysor is, on the one hand, influenced by higher cortical areas, and on the other, depends upon continuously changing proprioceptive signals. This latter, i.e. perception of the proprioceptive signals, was of main interest to us, and formed the chief object of our work on movement co-ordination disturbance mechanisms. The work was done at relatively low altitudes, where the

functional changes are not so striking but their influence on working movements and locomotion may be of great practical importance. Since the altitude factors have only a slight action, and individual reactivity differences were liable to obscure it completely (as several papers show). We arranged our work to analyse the data for relatively large groups of people under identical conditions.

Our investigations were carried out on the slopes of Èlbrus, and were mainly centred upon the Terskol peak (2,200 m above sea level) and on Priute II (4,200 m). The tests were carried out after 3–15 days at 2,200 m, and on the second day at 4,200 m, fatigue being avoided particularly by bringing the subjects up to 3,900 m on vehicles. The tests were performed in fitted chambers providing a favourable microclimate. The experimental subjects were typists, skiers and mountaineers, from which six groups totalling sixty people were formed.

The objects of our present investigation were mainly elementary movements, and simple forms of co-ordinated movement combinations involving few joints. This was because our primary aim was to study the relation between the motor analysor and the proprioceptive state, which can be revealed in an adequate and simple manner by very simple tests. A compound movement (in particular, working) is composed of, and co-ordinated by, elementary (voluntarily selected) movements. As will appear later, we studied the most important and also the elementary characteristics of the simplest co-ordinations—the delimitation of a volume, the maintenance of a uniform speed of movement within a set volume, and the maintenance of form in the final link of a two-joint system.

The form of movement selected as convenient for use under expedition conditions was the flexion of the middle digit at the metacarpus-phalangal joint. The arm was supported at the elbow in a convenient position, the forearm being at 20° to the horizontal with the wrist on a small plane table, the fingers hanging freely. A sleeve was fastened on the middle finger to prevent inter-phalangal movement. The task set involved smooth flexing against a small load (40 g) at a reasonable arbitary speed over a given range (30°). A kymograph was used for recording. A

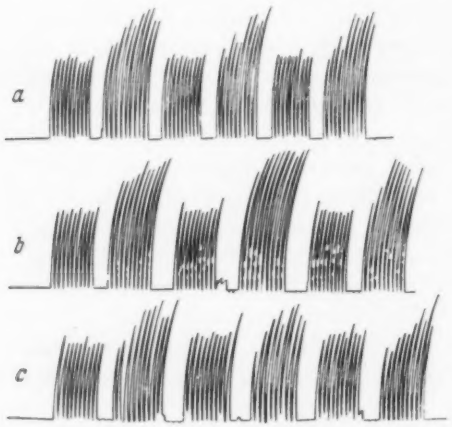

Fig. 1. Myograms of finger movements.
(a) at 2200 m. (b) at 4200 m on the second day. (c) at 4200 m on the fifth day.

series of movements were carried out under visual control (looking at the kymograph) and then with the eyes closed. Typical recordings are shown in Fig. 1.

Even at 2,200 m most of the movements (even some of those checked visually) exceeded the set range.

Another treatment of the data for 2,200 m (tests without visual control, Fig. 2) revealed that of 45 subjects 36 showed predominant movements, exceeding the set range by an average of 13 per cent; with the other nine the predominant movement decreased on average by 4 per cent.

At 4,200 m the number with predominantly increased movements rose to 41, the average increase approaching 20 per cent. The other four gave movements decreased on average by 4 per cent. Fig. 3 shows the changes in the percentage distributions of

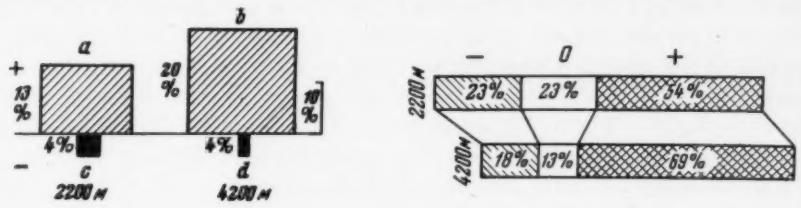

FIG. 2. Distribution of deviations in finger-bending myograms involving a set movement range: (a) 36 subjects. (b) 41 subjects. (c) 9 subjects. (d) 4 subjects.

FIG. 3. Shift in the distributions of insufficient, exact and excess finger movements.

reduced, exact ($\pm$ 2 per cent) and excess movements for all subjects with height. The sharp increase (from 54 per cent to 69 per cent) in the excess movements occurs at the expense of the others. We consider this result as indicating that the flexator impulses are either excessive, or last longer than is required to reach the desired angle and that this implies that the motor analysor is disturbed.

We selected the combination of elbow and shoulder joint movements involved in drawing circles through four given points at the ends of mutually perpendicular diameters, to analyse co-ordination in more complicated movements. The distances from the point where the diameters intersected (centre of a true circle) to the curve were measured along 16 radii, and the average result was drawn from the average value for each radius. Fig. 4 shows the true circle passing through the four points and the average "circles" from 14 tests at 2,200 m and 4,200 m. The tendency to "squaring-off" is already apparent at 2,200 m and is pronounced at 4,200 m.

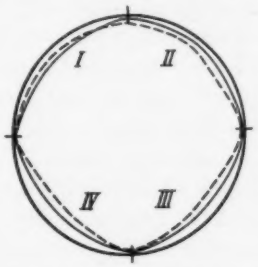

FIG. 4. Average circles drawn through four points for 14 subjects. Continuous line—2,200 m. Broken line—4,200 m.

The uneven distortion of different quadrants helped in elucidating the mechanism. The bio-mechanical explanation for each quadrant we offer (in a somewhat simplified form) is as follows. The first quadrant is drawn significantly, if not completely (in view of the variation in radial length) by congruent forearm movements with a single elbow position; for ease and simplicity of co-ordination it is sufficient that the curvatures of these arcs (of turning and elbow flexion) are directed in the same sense; so the quadrant is drawn by one movement of the elbow joint without any great compensating shoulder movement. The second quadrant mainly involves shoulder joint movement although a compensating bend in the elbow is required in maintaining a curvature corresponding to a radius less than that of the shoulder movement; as the shoulder moves out the elbow bends smoothly (this task is more difficult than drawing the first quadrant). Completing the third quadrant is still more difficult, since the bending arc at the elbow joint (which is leading the movement) is of opposite curvature to that of the quadrant being drawn; this requires a complex of compensating movements in the shoulder joint, as a smooth transition from outward to inward movement is needed. The fourth quadrant is no less complex, since the main movement is inward at the shoulder joint, but a smooth compensating transition from bending to straightening out is required at the elbow joint.

The symbols in Table 1 have the following meanings: *E* and *F* are principal movements in the elbow joint, *O* and *I* are principal movements in the shoulder joint, capitals implying principal movements and small letters compensatory movements.

TABLE 1

Joint	Quadrant			
	I	II	III	IV
Elbow	*E*	*F*	*F*	*fe*
Shoulder	—	*O*	*e—f*	*I*

E = extension. *F* = flexion.
O = outward *I* = inward

The arcs approximated straight lines in tests at 4,200 m, compensatory movements practically vanishing. If we substitute "correction" used in a physiological sense for "compensation" used in a technical sense, the mechanism of shoulder–elbow co-ordination disturbance can be formulated thus: the muscle co-ordination receptors and analysor are disturbed by height effects and correctly timed correcting impulses do not pass through; in consequence the required compound movement is degraded and carried out only by the primary effector muscles.

No less well defined is the disturbance of movement shown by carrying out tasks involving co-ordination of movement in the elbow joint with compensatory movements in the wrist (and partly finger joints). The task was to draw a straight line in a transverse direction between two points 25 cm apart with the shoulder in a fixed position. Seven subjects performed this test (12 times at heights of 2,200 m and 3,900 m). On several occasions quite straight lines were drawn (permitted error ± 1 mm), but on

others the lines were convex upwards or downwards (cases of S-shaped bends were assigned according to the predominant curvature). Eighty-four line-pairs were distributed as follows (see Table 2).

TABLE 2

Height (m)	Shape		
	⌒	—	⌣
2200	52	23	9
3900	61	22	1

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We see that it is normally difficult to draw a straight line, the lines being mostly convex. The instances of convexity are significantly increased at 3,900 m. The degrees of curvature as well as the number of convex examples increase; the average increase in curvature was 5 per cent. The convexity is due to the forearm acting as a radius; straightness depends upon a compensating movement in the wrist joint (which was sometimes excessive, giving a concave line). The increased frequency of convexity reflects the same co-ordination breakdown and loss of corrective movement as in drawing the circles.

A common way of illustrating (but illustrating alone) co-ordination disturbances produced by reducing the partial pressure of oxygen, is to have some phrase written; many such have been recorded in pressure chambers under conditions of extreme rarefaction, and they clearly show loss of the co-ordination involved in writing movements. But all the "explanation" of these illustrations does is to establish the existence of a disorder and correlate it with the "excited state of the nervous system". We have not found one single attempt to analyse the mechanism of this phenomenon.* We have confirmed that handwriting disturbances are noticeable only at great "heights" in a pressure chamber. Notes written at small "heights" are at first glance essentially indistinguishable.

At our suggestion several groups of climbers (82 in all) climbed Èlbrus, writing notes of the following standard format at 2,200, 4,200, 5,300 and 5,600 m: "I am writing this sentence at a height of (so many) metres." Signed. Date and time of day. All the scripts were measured from "I" to "metres". The indices used were the average letter heights and lengths; the number of connecting strokes between letters, and the stroke forms. Taking the data for 2,200 m as 100 gives the relations of Table 3.

Unfortunately the 5,300 m and 5,600 m scripts have to be omitted in spite of their telling nature, because conditions were so unfavourable (absence of a table, cold hands and fatigue); we only note that these results continue the tendencies found lower down, where conditions were extremely favourable (fitted warm room, complete relaxation before writing). The chief interest to us is the influence of relatively small heights, where the disturbance of motor functions was still subjectively undetectable, particularly after a rest. Table 3 shows that the data given

* This is the case with all such papers; from Tissand's notes made on a balloon flight to the latest work at our disposal, that of Govid and Luria [8], which was carried out in a pressure chamber.

Table 3

Height (m)	Letters			Form of stroke		
	Breadth	Height	Joining	⌒	—	⌣
2200	100	100	100	14	81	5
4200	104	122	82	19	79	2
5300	117	138	71	27	73	0
5600	127	198	61	36	64	0

by the common act of writing conform completely with that obtained above from the elements of graphs to which objections could be made on the grounds of artificiality. The increased letter widths and heights agree with increased finger movement amplitudes, and the changes in stroke forms completely coincide with the data from drawing horizontal lines. We derived a further index—joining strokes; their absence (letters contiguous) is very typical of the "illustrations", obtained at great "heights" in pressure chambers; this sign is clearly shown in our data for 4,200 m, and is of course due to our working with statistical numbers instead of single scripts. In origin this disturbance is undoubtedly linked with levels of abference, different analysis and integration other than those relating elementary co-ordination disturbances so far dealt with.

In this plan of notes there was some extremely interesting material present. The stereotyped notes were not adhered to in a significant number of cases, in one case at 4,200 m, and at greater heights. Transpositions ("This sentence I am writing"), replacements ("note" instead of "sentence"), forgotten and unfinished words and letters, or repetitions, mistakes in date and time, were all found. Notes containing additions such as "Europe is at my feet", "I write with shaking hand but Èlbrus is still before me" and so on, were extremely significant. This display of excitation in higher nervous centres at great heights (which has been repeatedly described) prompted us to study the simplest movements, dependent to a large extent upon the state of higher cortical centres.

The available results on the latent periods of simple motor-reactions are contradictory, this apparently being due to the tests used either being of short duration (pressure chamber, aircraft flight) or being obtained with professional guides, or from all sources—for single occurrences. In our tests the latent period was measured on 23 tourists at 2,200 m and 3,900 m (in both cases after the daily rest and under laboratory conditions on the same day). In 21 cases (91 per cent) the latent periods of a simple reaction increased (arithmetic mean of 20 results) on average by 23 per cent. In the remaining 9 per cent the reactions appeared to be unchanged.

The latent periods of reactions involving a choice showed an average lengthening of 11 per cent in 70 per cent cases. We only offer these data as evidence of an analogous tendency since we could not perform the adequate prior training required to ensure reliable quantitative results in work on reactions involving a choice.

The limiting frequency of middle finger movement (over a 30 sec period) was determined on 66 subjects by causing them to tap a key connected to a counter. At

3,900 and 4,200 m, 61 per cent showed an average drop of 7 per cent as compared with 2,200 m, 7 per cent showing a drop of 7 per cent, and 22 per cent no change.

We also attempted to study the reflex time in the following way: for 20 sec the subjects (38 in all) had to respond to the beats of a metronome repeated once per second by striking a key, and had to continue the operation for a further 20 sec at the same speed after the metronome was stopped. Of the 38 subjects, 21 showed a decreased tempo at 3,900 m as compared with results obtained at 2,200 m; nine showed an increase, and eight no change. Although this test still requires that we mention methodological development because it discloses a tendency towards retarded motion even at 4,200 m.

We certainly could not consider exhaustively the conclusions drawn in published work, which amount to the statement that "the writing became illegible" when we consider our "standard notes" and the published handwriting tests. We found changes in letter sizes and stroke curvatures while the writing was still legible. In principle it is caused by distortion of the shapes of letters taken as distinct graphic signs; failure to close ovals, bending vertical strokes, omission to complete individual letters, etc. A true legible performance of all these details requires a delicate and well perceived co-ordination of wrist muscles, mainly via automatic cut-in and cut-out of antagonists—bending and straightening the fingers. The disturbance of proprioception is thus shown by the decrease in legibility of an individual's writing. Even a "printed" letter may to a certain extent be considered as a sign that the continuity in the proprioceptive signals is destroyed. We are satisfied that the characteristic elements of illegibility were present and did not perform the extremely laborious task of measuring the parts of letters in notes written at the various heights. In our arrangement a motor function was tested on which we were able to detect and measure disturbances on delicate movements at quite low altitudes—i.e. bending the third finger. The record on a fast-moving kymograph can hence be used to study the smoothness and hence the controlability of this elementary movement. A bending kymogram (Fig. 5) is of single-phase type and has an initial acceleration and a final deceleration. Lack of smoothness is shown by additional waves of acceleration and deceleration, these sometimes giving a jagged appearance; this reflects ill-matched pulse trains and intermittent brief interruptions, followed by compensating increased pulse trains.

Not all 39 subjects or all of 10 successive movements were smooth normals (at 2,200 m). "Perfect" movements amounted to 26 per cent of the total (at 2,200 m); at 4,200 m this fell to 13 per cent. The number of faulty movements (wavy or indented) increased in 27 subjects, in 4 it remained the same, and in 8 it decreased.

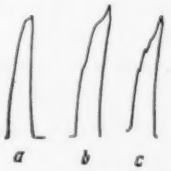

FIG. 5. Myograms of finger flexion and extension taken on a fast kymograph.
(a) Smooth flexion. (b) Flexion involving speed variations. (c) Interrupted flexion.

While the number of imperfections increased at 2,200 m, the only faults were waviness the speed being variable but unidirectional; while at 4,200 m many disturbances were of sawtooth types, which involves discontinuous forward motion. Fig. 6 illustrates this.

Our factual summary quite clearly demonstrates co-ordination disturbances at relatively low altitudes when extraneous effects (fatigue, cold, etc., are eliminated) and subjective perception of disco-ordination is absent. We here undoubtedly encounter complex combinations of various disturbance mechanisms even with these simple tasks.

Consider the task of maintaining a given range of finger movement. The range increase may be considered as a particular expression of a general excited state. But the task was subject to constant self-checking (groups of movements checked visually); but when visual checks were eliminated the range increased. We noticed one important detail. The increase in range was not always of staircase type, which might be

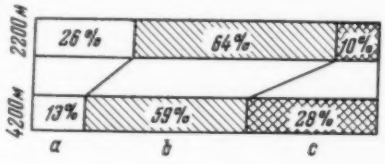

Fig. 6. Distribution of all myograms over (a) smooth, (b) uneven and (c) interrupted styles.

related to fading in the proprioceptive traces from movements; an increased range and "staircases" developed quickly and in any order when visual checks were eliminated. So here we have a defect in the afferent reflex link with proprioception continuously disturbed as well as residual reflex fading due to general excitation. Hence the excessive movements must be explained by the proprioceptive impulses which must indicate the end of movement being inadequate (or inadequately perceived) and the effector pulses being excessive in size and duration. In other words inhibitory processes either in the proprioceptors or in the motor analyzor appear here.

The hand biomechanics involved in drawing visually checked lines and circles considered above discloses the basis of an inhibitory state already existing in the integrating effector zone which is expressed by a falling off in correcting impulses.

In our opinion this is the origin of "illegibility" in writing.

Thus all links in the motor apparatus except the peripheral effector are somewhat inhibited at 4,200 m. We were not able to carry out special tests on the effector link under the expedition conditions at 4,200 m, and saw no need for them since it is very doubtful whether muscles are capable of responding to voluntary impulse series around rates of 250–300 min^{-1}, as occur in experiments on the limiting rates of motion. The reduced limiting rates of voluntary motion observed did not indicate the muscular state, but disclose the ease with which the central effector link becomes inhibited. The increased reflex times in most subjects indicate tendencies to inhibition in the higher cortical centres. In passing we note that attention is only devoted to signs of excitation in most work.

The data at 4,200 m show that a complex of factors causes a non monotome changes in voluntary actions in man, primarily destroying central co-ordination and

harmonious relations between the functional central nervous areas. The over-excitation of some cortical areas due to inadequate external stimuli is even accompanied by inhibition in other areas. Roughly speaking the higher centres are excited and to some extent disorganized, and the motor analyser, according to all our data is largely inhibited. Various motor phenomena related to the excited states in the higher centres may appear on the background of this induced inhibition, which also confirms the presence of signs of disorganization in all nervous activities.

The state of the motor analysor reflects directly on the evaluation of proprioceptive signals received in making a movement as these signals define the type of movement and the accuracy of the final result.

Our analysis leads to the conclusion that subjectively inapparent co-ordination disturbances are revealed by objective methods at the relatively low height of 4,200 m which arise mainly from inhibition in the motor analyser.

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We must pause on an important detail. All the observations at 2,200 m were carried out after a day's stay at this level, while the data for 4,200 m were taken after several days' stay. Then our data demonstrates the clear-cut influence of height freed from physical strain effects. Undoubtedly the effects found in low levels are transient. This is actually confirmed by the limited but reliable data we obtained with a group of skiers (18 subjects) repeatedly studied on the fifth continuous day at 4,200 m, using range, smoothness, rate maintenance in movements of the third finger, and handwriting tests. The number of excessive finger movements completely returned to normal (Fig. 7). The limiting rate reached 97 per cent of normal, and weak residual disturbances could be demonstrated in delicate finger co-ordination (test of smoothness and writing). The positive significance of such recovery is that the acute co-ordination disturbance (at 4,200 m) are transient. The important point is that the duration of rest between stages (securing equilibrium and the adapted state required for further climbing) is still not determined by physical fatigue and the cardio-vascular state, since the central nervous disturbances (particularly those in its sensomotor functions) were found to be clearly developed on the second day's stay at quite a low altitude.

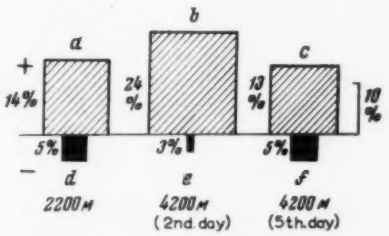

FIG. 7. Distribution of finger-movement-range deviations in a group of 18 subjects remaining at 4,200 m for five days.
(a) 13 subjects. (b) 17 subjects. (c) 13 subjects. (d) 5 subjects. (e) 1 subject. (f) 5 subjects.

The data obtained on the acute movement co-ordination disturbances and adaptations at 4,200 m would appear to us to show that these should be borne in mind when designing ways of performing tasks of short duration in high mountain conditions, e.g. on expeditions, geological prospecting, and by mountaineering parties.

CONCLUSIONS

(1) When 60 subjects adapted to a height of 2,200 m for periods between 3 and 15 days were studied on the second day at 4,200 m, co-ordination disturbances were found in 75 per cent. Slight residual effects were found on studying 18 on the fifth day.

(2) The co-ordination disturbances were shown by the subjects' exceeding a set range in an elementary flexing movement, by the uneven speed of movement and by the absence of correcting components in complex movements (drawing lines and circles, writing).

(3) The latent periods of a simple motor-response were increased (in 91 per cent) and the limiting rate of motion reduced (in 61 per cent) simultaneously.

(4) The typical signs of excitation and disco-ordination in the higher cortical centres were observed (euphoria, unjustified associations, emotional state, etc.).

(5) The type of disturbance denotes that the motor analysor is inhibited: analysis of proprioceptive signals being slow and inadequate.

(6) Motor analysor inhibition can be explained by negative induction from the excited higher cortical centres.

Translated by G. W. G. Sharp

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ACTION POTENTIAL PICK-UP FROM SINGLE GANGLIONIC RETINA CELLS USING THE MICROELECTRODE METHOD

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(*Received* 15 *October* 1956)

The action-potential pick-up method for single retinal elements developed by Granit and Svaetichin [1], occupies a significant place among the various microelectrode methods of detecting single nerve cell and fibre potentials. This procedure has a number of special features, whilst being a particular intracellular potential pick-up method. Much work has been done on perfecting the microelectrodes and the actual conditions of retinal potential pick-up have been studied [2, 3, 4, 5] since the method

was first used. But all is not yet clear; so, for instance, Barlow [5], who determined the optimum microelectrode-cup shape, empirically concluded that the microelectrodes were often still unsuitable although fulfilling all the necessary demands.

The present work was undertaken to elucidate some conditions of retinal-potential pick-up not considered in earlier investigations, and to provide a most favourable microelectrode design.

The normal microelectrode used on the retina has a metallic core (of platinum or silver, with a 20–30 μ tip) insulated right to the electrode tip from the surrounding medium. Only the transverse section of the metallic conductor, or just its tip, comes in contact with the tissue. The microelectrode is brought up to touch the inner retinal surface facing the vitreous humour. The other electrode is usually placed anywhere in the vitreous humour or on the eyeball. Both electrodes are connected directly to the a.c amplifier input. The moment of contact between electrode and retina is shown by a certain increase in the noise level and by the appearance of action potentials in response to light stimuli. By moving the electrode slightly in a lateral direction and pressing it gently towards the retina, an optimum position is found at which the impulses vary in frequency but remain more or less constant in amplitude.

Several properties determine the suitability of a microelectrode for detecting action potentials. This primarily relates to the electrode resistance which depends on the metal used, on the diameter of the internal conductor, etc. Barlow [5] pointed out that it was important for the electrode–retina contact to be a flat surface. Our work also showed that thickness of the glass insulating-wall of the electrode also plays an essential role. Electrodes with internal diameters at the tips of some 20 μ and thin glass walls (1–5 μ) were found to pick up the pulses poorly, or not at all. On the other hand electrodes of exactly the same internal diameter but with a slightly greater glass thickness (40–50 μ), were found to work much better.

Let us now consider the significance of the above factors in some detail.

1. IMPORTANCE OF THE INSULANT THICKNESS AND OF THE ELECTRODE CONTACT SURFACE

Several workers have shown [2, 5] that the impulses picked up by the microelectrode are identical with the action potentials of retinal ganglionic cells. The activities of nerve fibres situated over the ganglionic cells and coming in direct contact with the electrode are usually undetected. Even if such impulses are detectable, they are unstable and it is very difficult to isolate the response of a single fibre from those of neighbouring ones [5].

Ganglionic cells are situated at some distance from the surface (15–30 μ). Since the microelectrode does not penetrate the retinal tissue and is only pressed closlye to its surface, the pulses picked up are not actual cell-potential changes but changes in the electric field created in the surrounding medium. The potential changes detected will depend on the electrode–retina separation and on the field configuration. As a medium with an electrical resistance much lower than that of nerve tissue (vitreous humour or physiological liquid) is always close to the potential source, the nature of the field is such that the potentials at various points on the retina are similar and are of the same order as that of the vitreous humour (or of the physiological liquid); the second "neutral" electrode is, in practice, always connected with this.

A microelectrode in contact with the retinal surface should not register any potential at all under such conditions, provided the electrode itself does not cause any essential changes in the field distribution. In reality, an electrode with thin insulating glass walls is capable of removing pulses of only very small amplitude which only slightly exceed the noise level. This is because the region lying directly under the electrode tip is shunted by the surrounding liquid via the thin layer of nerve tissue which lies under the insulating wall.

An increase in the wall thickness modifies the pick-up conditions. If this type of electrode (with increased wall thickness) is brought into contact with the retina, the conducting liquid is squeezed out from underneath, the shunting is reduced and the potential under the electrode (and consequently also the potential picked up) increases.*

Hence the electrode sheath not only insulates the conductor from the surrounding liquid but also keeps the liquid away and prevents shunting at the pick-up point.†

This shows why it is so important to ensure an end-surface to the electrode as flat as possible: if the liquid penetrates to the point of contact via irregularities in the face the pick-up point is shunted and the amplitude of the pick-up pulse is reduced.

2. MICROELECTRODE RESISTANCE

One of the most important properties of an electrode is its resistance. The amplifier noise level increases with the resistance and so the potential changes become less detectable, and if the electrode resistance approaches that of the first input stage, much of the potential (depending on the resistance ratio) will be lost in the electrode. Since the potentials picked up by intracellular electrodes are comparatively small the amplifier noise-level must be minimized. The electrode resistances were measured here by two methods (Fig. 1).

In *A*, the resistance R_2 was selected to be such that when connected to the input

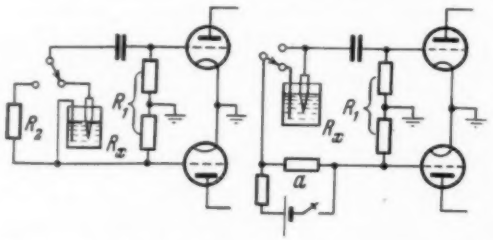

FIG. 1. Circuits for electrode resistance measurement.

it gave the same reduction in noise level as was obtained with the electrode placed in physiological saline. Then $R_x \approx R_2$.

*A more detailed or a "quantitative" dependence of the electrode-removal conditions on the thickness of the electrode-insulating wall will not be considered in the present work.

†An alternative reason why electrodes with thin insulating walls are unsuitable could also be put forward. The capacity between metal and surrounding medium increases with decreasing insulant thickness the time input constant, thus also increasing the upper limit of the pass band being reduced. However, a simple calculation shows that, even with an electrode resistance of 1 MΩ, a significant distortion in impulse amplitude could be expected only with an input capacitance of 100 pF or more. The capacitance of the electrode itself, on the other hand, does not exceed 10 pF even if the wall thickness is about 1μ.

In *B*, rectangular current pulses from a low-resistance calibrator *a* were applied to the input via the electrode (placed in physiological saline) and a low-capacity condenser (5,000 pF) to reduce polarization. The oscilloscope deflections were compared with those derived from standard pulses fed direct to the amplifier, the electrode being cut out. The deflection differences depend on the potential drop in the electrode resistance. The electrode resistance is then readily calculable knowing this difference and the first stage input resistance R_1.

The resistances of our electrodes using platinum wires with 20 μ tips were found by the above two methods to be ≈600–800 kΩ. Pick-up by these electrodes was quite good [6]. But when we consider the input resistance (here 500 kΩ), we see there is still a considerable potential loss in the electrode. Increasing the input resistance effects no improvement because the amplifier noise-level increases. The resistances exceed 1 MΩ with 10 μ wires; the action potentials only slightly exceed the noise level [3], if it is picked up at all, when such electrodes are used.*

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What actually determines the electrode resistance?

The overall resistance of an electrode placed in saline has three components: (1) the metallic core, (2) the liquid hemisphere round the electrode tip and (3) contact resistance between liquid and metal. (1) (~ several ohms) can be neglected, (2) can be calculated to be about 15–20 kΩ for 20 μ tips. Consequently all the rest is the contact resistance between metal and liquid.

Note that our values differ considerably from those reported for microelectrodes. Thus, Bralow [5] gives a value of only 15 kΩ (approximately) for a 20 μ platinum electrode, immersed in the saline appropriate to cold-blooded animals, so his contact resistance was very small. Svetikhin [3] and Gray and Svetikhin [4] earlier gave about the same value. Their electrodes had diameters down to 0·5 μ and their total resistances were about what would be expected from the metal–liquid contact alone.

But Svetikhin and Gray [3, 4] only obtained these values with special electrodes. Svetikhin states that, if ordinary solder, silver or gold are used instead of platinum, their resistances increase considerably because low-conductivity layers form on the surfaces. The silver solder at the tip is therefore rhodium-plated (rhodium being a platinum-group element) and then coated with platinum black. Even then the slightest surface contamination increased the resistance considerably. The contamination can be removed with chromic acid, or if this is insufficient, by additional platinization. This procedure had to be followed before each resistance determination (in saline) otherwise the value found might be much higher than expected. Gray and Svetikhin give the following figures to illustrate this: 14 MΩ before and 0·336 MΩ after cleaning since contamination due to bringing the platinum surface into contact with tissue is very likely, and so the resistance is likely to be unstable, we can immediately see why Svetikhin's electrodes are not the best ones. In addition, the possible advantages of these electrodes (comparatively low resistances with very small external diameters) can only be used if they are inserted in the tissue, which, as already mentioned above, is not absolutely essential in order to pick up action potentials of retinal ganglionic cells.

Our attempts to reduce the platinum–liquid contact resistance by cleaning the

* Bongard and Smirnov have found the same sort of values for platinum electrodes about 20 μ in diameter (verbal communication).

electrodes with chromic acid were not successful. It would appear that, apart from platinum, the nature and freshness of the cut surface play a large part, as does the purity of the platinum. This may explain the considerable discrepancy between our results and Barlow's, since Barlow would appear to have cut the platinum freshly before inserting the electrode into the liquid (his electrode was a 20 μ platinum wire, coated with varnish).

We used another approach in dealing with electrode resistances. Since the main resistance is attributable to the liquid–metal contact, it was essential to increase the interfacial area without altering the contact area between electrode and tissue. The platinum was replaced by a column of liquid for this purpose, or in other words, a liquid microelectrode was used instead of a metal one. A 100 μ platinum wire was only inserted into the wide part of a glass capillary tube filled with saline. This arrangement gave a metal–liquid contact surface so large that the contact resistance could be neglected. The main resistance of such an electrode is due to the column of liquid in the narrowest part. A marked reduction in resistance can be produced by making the constriction as short as possible and by replacing the physiological saline in the electrode by more concentrated saline. A successful liquid electrode with a 5–8 μ hole, filled with a 10 per cent solution of NaCl (in agar), had a resistance of about 60 kΩ when placed in saline appropriate to cold-blooded species. Calculation shows that this resistance is mainly that of the liquid hemisphere round the electrode aperture, and so the resistance of the electrode itself is only a small fraction of the total.

3. PRODUCTION OF MICROELECTRODES

Hence the main demands which a microelectrode to be used for detecting action potentials of retinal ganglionic cells must satisfy are, firstly, that the resistance should be sufficiently small when the internal diameter is about 20 μ (< 100 kΩ) and, secondly, that the part in contact with the tissue should be surrounded by an adequately thick insulating layer (< 30–50 μ), and that the transverse sections should be as smooth as possible.

The following method of preparing microelectrodes conforming with the above may be proposed.

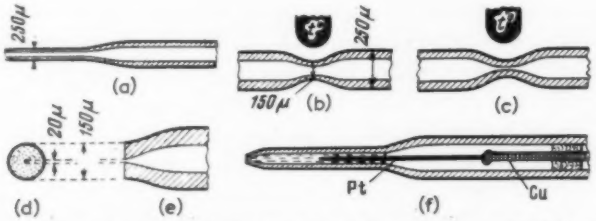

FIG. 2. Stages in preparing microelectrodes for picking up action potentials in ganglionic cells; t^0 is an electrically-heated platinum loop.

A thick-walled capillary tube of diameter 2–3 mm is drawn out at one end to a thickness of 200–300 μ (Fig. 2a). The capillary is placed under a microscope and an electrically heated platinum loop (wire diameter 300 μ) is placed round the thin section—as in Fonbrun's microforge [7], and its thickness reduced to 100–150 μ at one point by slight stretching (b). The thin section is further heated without pulling,

the lumen diameter being thereby further reduced (c). The capillary is now broken off at the narrow neck and the fracture surface smoothed on very fine sandpaper or on a whetstone until the fracture surface is perpendicular to the electrode (e). The tiny fragments of glass or quartz thereby pushed into the capillary are best removed with a steel wire ground down to a thickness of 10–15 μ at one end. When the polished surface is examined under a binocular microscope (d), a small hole is seen; the diameter of which can be easily determined with a micrometer ocular.

The thin part is now immersed in 10 per cent NaCl solution, and the capillary fills with solution. It is convenient to add 3 per cent agar to the NaCl solution and to use it hot for filling the electrode. To reduce the rate of electrolyte dilution at the electrode tip by the physiological saline and to prevent the concentrated solution flowing into the surrounding liquid or tissue. Finally, a platinum or silver wire is inserted in the wide end of the capillary to ensure good contact with the liquid inside the capillary (f).

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The resistance of such an electrode (hole diameter ~20 μ) placed in cold-blooded species physiological saline is usually 80–100 kΩ, and if necessary this can be still further reduced (by increasing the sharpness with which the lumen is constructed). A low resistance electrode of this type has already been instanced above.

The electrodes are stored in 10 per cent NaCl and only removed during experiments. Ion diffusion through the agar during an experiment (1–2 hr) is usually so slight that the electrode resistance scarcely changes. The same electrode is used repeatedly.

The desire to detect from single ganglionic cell action potentials from the retina normally implies that the size of the active electrode surface must be reduced as far as possible. The response from a single cell is actually easier to detect with a 20 μ electrode than with one of 40 μ. But there is no point in having electrodes with internal

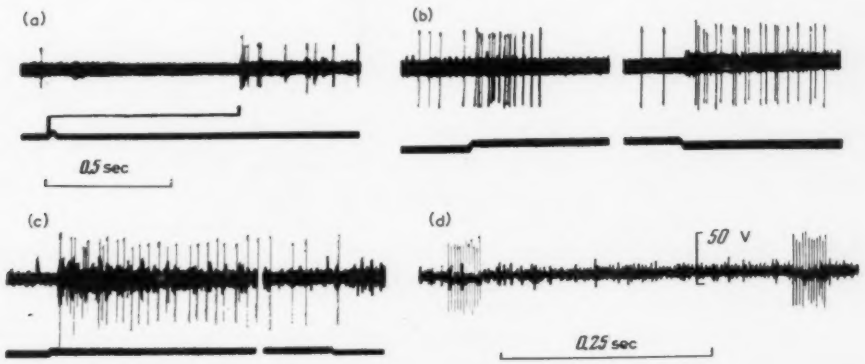

FIG. 3. Examples of action potentials picked up with a microelectrode.
a—c are impulses from the single retinal ganglionic cells of various animals; the top lines are oscillograms, the lower lines denote light stimuli: time base for all the three curves is 0·5 sec; a—frog retina pulses: response to a single flash followed by the cut-out effect. The line under the oscillogram denotes the latent period. b—retinal pulses from a cat: cell showing cut-in and cut-out effects; c—retinal pulses from a guinea-pig: cut-in effect; d—pulses by a microelectrode inserted to a depth of 4 mm into the cornea of a rabbit: the pulse source is evidently a damaged neurone. Approximate scale for all curves, 50 μkV.

diameters less than 15–20 μ. The reason is that the electric fields of adjacent ganglionic cells overlap quite considerably, and each section of the retinal surface, no matter how small, lies in the electric fields of several ganglionic cells. So, even if very small how small, lies in the electric fields of several ganglionic cells. So, even if a very small electrode diameter is used to detect single-cell responses, some spot on the retinal surface where the response of a particular cell is much greater than those of its neighbours must be found.

Fig. 3 shows action potentials recorded with a microelectrode from ganglionic cells in frog, cat and guinea-pig retinas. The amplitudes from the frog are much less than from the two mammals, which may be due either to differences in the properties of the ganglionic cells themselves or to differences in the distances between the cells and the inner retinal surface. Fig. 3d shows that these microelectrodes can be used for detecting pulses from nerve cells in certain other structures, and, in particular, for detecting pulses from neurones in the visual radiation zone of the rabbit cortex. Of course the optimal electrode shape selected will depend on the specific conditions obtaining in any particular investigation.

Translated by H. CYGIELSKI

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AN ACID ERYTHROGRAM METHOD

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(*Received* 20 *June* 1956)

THE differential investigation of leucocyte composition is a necessary part of clinical and experimental blood analysis. The many-sided possibilities and utility of this method are well known and need no proof.

The red cells are of no less importance in blood, their content exceeding that of leucocytes by a thousandfold, and while they undoubtedly have a physiological heterogeneity they are usually studied only *en masse* (erythrocyte count, haemoglobin content). The existing qualitative methods of red-cell analysis—counting of reticulocytes and other morphologically distinguishable forms, visual erythrocytometry—are not widely used on account of technical difficulties and their limited and obscure physiological significance. Hence the search for new differential methods of erythrocyte investigation remains an important physiological problem.

The simplicity of the micro-structure of the erythrocyte enables one to differentiate qualitative red-cell pictures predominantly from the point of view of physico-chemical erythrocyte properties rather than morphologically.

The co-operation of many physico-chemical properties of the erythrocyte manifests itself in the stability of the erythrocyte against destructive agents. Thus the index of erythrocyte stability may be used to specify their state. The development of accurate objective photoelectric methods of analysis has made the study of erythrocyte stability easy.

The method most widely used in the past for study of erythrocyte stability is the "two-point" method—maximum and minimum resistance. The name itself implies that it is not a differential method, since it determines only the beginning and end of the haemolysis process, and not the distribution of erythrocyte stability throughout the population.

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A number of new methods has been proposed in recent years using erythrocyte stability for the differentiation of their condition [1–8]. Following the "two-point" tradition, these methods are mainly based on hypotonic haemolysis. In particular, the osmotic erythrogram method uses photoelectric recording of degrees of haemolysis in a series of solutions of diminishing tonicity [9].

Use of erythrograms in physiological [10] and in clinical work [11] has shown that osmotic erythrograms give a detailed picture of the qualitative red-cell composition, and are sensitive to physiological and pathological changes of erythrocyte condition. At the same time some technical defects inherent in hypotonic haemolysis still occur in osmotic erythrograms. This is due to the circumstance that a number of different blood samples are used to construct the osmotic erythrogram (not less than 8–10 solutions being used for the hypotonic series). This reduces the accuracy and increases the difficulty and demands on the worker's technique. This method requires an amount of blood (0·3–0·5 cm^3), sometimes difficult to obtain without venepuncture.

This difficulty can be avoided if we no longer use the "step-wise" measurements of the hypotonic method, but the kinetics of the haemolysis, a time-series being taken on one blood sample. The use of haemolysis kinetics requires a different haemolytic agent, since hypotonic haemolysis is too fast, and is difficult to follow. In this connection we should remark that there is no proof of special physiological advantages in using osmotic stability, as opposed to stability towards other haemolytic agents, as an index of the state of the erythrocytes.

A large number of haemolytic agents, both physical and chemical, can be used in suitable concentrations so as to adjust the haemolysis time within wide limits.

The kinetic method makes heavy demands on the stability of the haemolytic agents. The ones widely used—saponin, digitonin, etc.—are not satisfactory, being alkaloid mixtures of variable composition that hydrolyse rapidly in aqueous solution.

The need to prevent access to air, limits the practical use of alkaline solutions.

Simple inorganic acids produce the most stable haemolysis. Of these HCl comes closest to the ionic composition of plasma.

The haemolysis time is linearly related to the concentration of the haemolysing agent. The most convenient concentration for normal human blood was 0·002 N HCl, this taking 7–8 min.

In practice, the acid erythrogram is taken as follows: 2 mm³ of blood suffice for one analysis, this being diluted to 1:1000 with physiological saline (extinction 0·700). The erythrocyte suspension remains usable for analysis on keeping for 3–4 hr at room temperature. The storage times for animal bloods are different.

An FEK-M photoelectric colorimeter was the basic equipment used in measuring haemolysis kinetics.

The left-hand beam passed through a 20 mm cuvette, double-walled, water from a thermostat being circulated through the jacket. A blank cell, containing water, was placed in the other beam.

To set the left-hand control at zero, with the working cuvette filled with physiological saline and with a red filter, the galvanometer needle was set at zero. The saline in the working cell was replaced by 2 cm³ of the uniformly mixed suspension of erythrocytes. 2 cm³ of the working haemolysing solution (0·004 N HCl) were added as rapidly as possible and mixed. The acid concentration in the cell was thus 0·002 N. The start of haemolysis was taken as the time of adding the haemolyser. The reading of the extinction on the left-hand cylinder was taken every 30 sec. A Warrens motor (2 rev/min) was used as time-marker, this giving an audible signal every 30 sec. Measurements were continued until two successive readings were identical, i.e. haemolysis was complete.

A falling series of extinctions was thus observed, each corresponding to the degree of haemolysis at the time of reading. A number of conditions must be observed in order to obtain consistent results:

(1) The reaction temperature must be constant, since the haemolysis rate is related to the temperature of the solution. For a number of reasons a temperature of 24° was chosen. The temperature was kept constant by carrying out the reaction in the temperature-stabilized cell, and by placing the blood sample vessels containing the erythrocyte suspension, the reagent and the pipettes, in the thermostat that fed the cell.

(2) The haemolysis rate also depends on the concentration of the haemolyser. This was therefore prepared from pure HCl, diluted to 0·004 N with physiological saline (0·85 per cent chemically pure NaCl) and checked by titration.

The phenomenon on which the fall in extinction during haemolysis is based naturally comes into question.

There are two possibilities: either the haemoglobin escapes gradually from all the cells, this being completed more or less simultaneously, or else the cells are of different stabilities and break up in a sequence determined by the distribution of these stabilities. In the second case, the break-up of each erythrocyte may be supposed rapid, being completed in a time less than that between readings of the extinction. In the first case, the fall in the extinction represents the kinetic gradual loss of haemoglobin from all the cells. If this is a correct theory of the mechanism, this method is of limited physiological value. The second theory implies that the fall of extinction reflects the sequential haemolysis of cells of greater and greater stability. In this case the result is of major physiological importance, since the cells can be differentiated as to their stabilities.

In order to discover which of the two reactions is operative under our conditions, photomicrographs of the cells were taken simultaneously with the recording of the

erythrograms. Part of the mixture was taken from the cuvette directly after mixing with the haemolysing acid and placed in a counting chamber previously fitted with a camera, and the cells were photographed at the instants of taking the extinction readings. A mirror camera and cine-film (exposure 0·02 sec) were used. The temperatures of the chamber and the cuvette were kept the same.

Comparison of the photograph showed a regular drop in the cell count from frame to frame. From this it follows that the cells in a given blood differ in stability to HCl, and do not break up simultaneously. The erythrocytes can thus be differentiated according to stability by means of the haemolysis kinetics.

To establish the relation between the photometric measurements and the true cell break-down, the erythrocytes disappearing from successive frames were counted, and the result compared with the optical density changes in the same period.

Analysis of a series of photomicrographs showed that the cells began to disappear 1·5–2 min after the start. Thus the break-down of the first cells corresponds to the extinction changes beginning at 1·5–2 min: the earlier extinction changes are evidently due to prior changes in the cell shapes (spherulation).

The interval between extinction readings was chosen by reference to photographs taken at 10 sec intervals. When adjacent frames were compared it was seen that an erythrocyte which was clearly visible and intact in one frame had vanished in the next one taken 10 sec later, i.e. the time required to haemolyse an individual erythrocyte did not exceed 10 sec. Hence an interval of 30 sec between readings ensures that it is impossible to count a given cell twice.

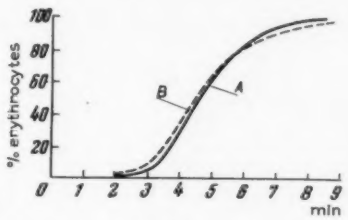


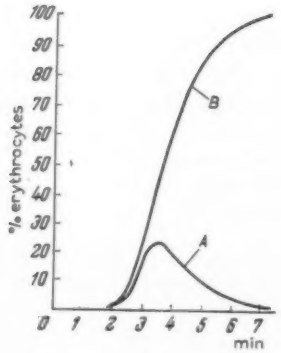
FIG. 1. Haemolysis kinetics in 0·002 N HC1. A—computed from the photomicrograph; B—photometric curve.

For convenience in comparing the photometric and microphotograph results, all data were expressed as percentages of the initial values. Fig. 1 shows the results of one such experiment. Curve A was obtained microphotographically and B photometrically. The shapes of the curves A and B are in general the same. From this we may conclude that the photometric curve reflects the haemolytic process and corresponds to the distribution of resistance among the erythrocytes. The change of extinction from the actual start of haemolysis to its completion is proportional to the total number of erythrocytes participating in the process. This assumption is confirmed by the studies of the optical properties of an erythrocyte suspension on which the photometric method of cell-counting is based [12]. Thus the percentage distribution of erythrocytes by stability may be computed from the extinction curves. Table 1 gives figures from one such experiment with human blood to make clear the calculation.

TABLE 1

Duration of haemolysis, mins, T	Extinction, E	Change of extinction, ΔE	Erythrocytes haemolysed, H %	$i = n$ Σ, Hi, % $s = 1$
0·5	0·420	[0·007	—	—
1	0·413	0·005	4·5	—
1·5	0·408	0·004]	—	—
2	[0·402]	0·002	0·6	0·6
2·5	0·400	0·019	5·3	5·9
3	0·381	0·070	19·5	25·4
3·5	0·311	0·082	22·8	48·2
4	0·229	0·062	17·3	65·5
4·5	0·167	0·047	13·1	78·6
5	0·120	0·031	8·6	87·2
5·5	0·089	0·022	6·1	93·3
6	0·067	0·013	3·6	96·9
6·5	0·054	0·008	2·8	99·2
7	0·046	0·003	0·8	100·0
7·5	0·043	0·000	—	—
8	0·043	—	—	—
	0·359	0·359	100·0	—

The first and second columns in the table show the duration of haemolysis (min) and the corresponding extinctions. The third column gives the differences of extinction ΔE between successive readings. This represents the number of cells broken during the interval between two readings, i.e. is a quantitative measure of



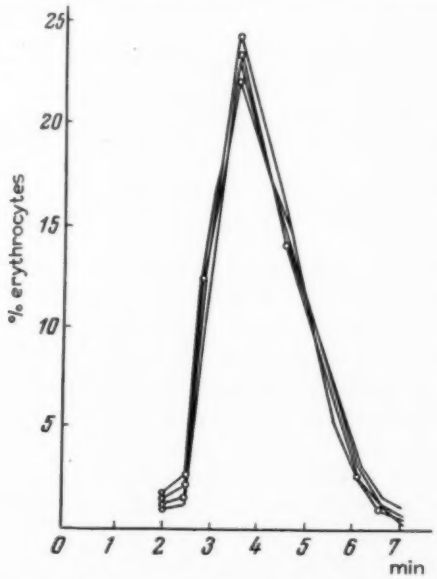


FIG. 2. A—erythrocyte stability distribution curve; B—haemolysis, %.

FIG. 3. Four curves taken with the same blood sample.

the erythrocyte group whose stability is expressed by the time from the start of haemolysis to the given reading.

The value of ΔE is seen to be least during the second minute, i.e. the change of extinction slows down at this time. The microphotographs showed that this minimum is due to pre-lytic changes in erythrocyte shape during the first $1\frac{1}{2}$–2 min, accompanied by considerable changes in the scattering of the light. This is recorded by the apparatus and the extinction readings change. This change had usually ceased by the second minute (under our conditions), and ΔE was a minimum: the extinction changes produced by spherulation are enclosed in square brackets in Table 1. The least stable cells then begin to break down, and ΔE again rises. The subsequent drop in extinction is due to the falling off of the number of erythrocytes in the sample, and ceases only when the last cells are broken down. In practice, identical readings are found from this time on.

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It is noticed that the extinction does not fall to zero at the end of the haemolysis. The residual extinction is due to absorption of light by the haematin hydrochloride formed from the cell haemoglobin by the haemolysing acid.

The difference between the extinction at the minimum value of ΔE and that at the end of haemolysis is taken as 100, and in the fourth column of the table the values of ΔE are expressed as percentages of erythrocytes of given stability (H per cent) in column 4. This makes the results independent of the red-cell count and the haemoglobin content.

The percentage distribution of erythrocyte stability is conveniently drawn as a graph relating the percentage of H to the duration of haemolysis, this being termed an erythrogram. Fig. 2 shows one such (curve A), plotted from the results of Table 1. Curve B of Fig. 2 shows the haemolysis process: it is drawn from the figures in column 5, which show the increase in percentage haemolysis with time.

When the appropriate conditions are observed, the erythrograms are stable and reproducible: Fig. 3 shows the curves from four samples of the same blood specimen.

Analysis of the erythrograms for healthy individuals showed that the erythrocyte stability distribution is very constant in normals. The maximum is found at 3·5 min, the beginning of haemolysis at 1·5–2 min and its end at 7–7·5 min under the above-described conditions are observed. The distribution curve is asymmetric. The average of the erythrograms from 50 clinically normal individuals is shown in Fig. 4. The mean deviation is shown by the broken lines.

When the equilibrium of the blood system is disturbed, deviations of the erythrocyte stability distribution from normal occur. Examples of such pathological changes are given in Fig. 5.

The changes in the erythrocyte stability distribution (relative to normal) may be specified in different ways. One convenient characteristic is the stability index, computed from the formula,

$$I = \frac{\%\ H_n.t_1 + \%\ H_2.t_2 + \ldots\ldots + \%\ H_k.t_k}{\%\ H_{n1}.t_1 + \%\ H_{n2}.t_2 + \ldots\ldots + \%\ H_k.t_k.}$$

where the percentage of H_n.t is the product of the percentage of H for the given stability group and the haemolysis time, for normal blood, and k = 1, 2, 3 . . . , and the percentage of H.t is the same expression for the blood under study.

Table 2 gives for the mean erythrogram of 50 normals, the product of the percentage of erythrocytes, and the corresponding haemolysis time.

The stability index is 1 for the mean curve. The curve is displaced to the right

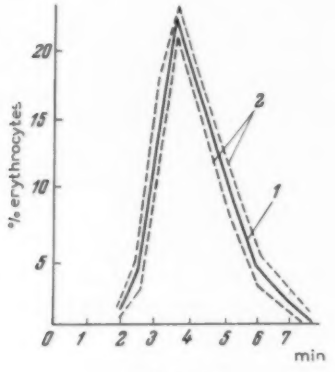

FIG. 4

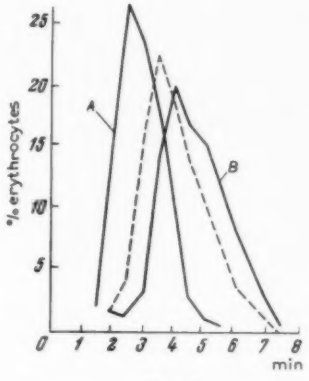

FIG. 5

FIG. 4. 1—Average erythrogram for normal human blood; 2—limits of variation.

FIG. 5. Pathological erythrograms.

A—Patient *B*. Diagnosis: acute gangrenous appendicitis, second day after operation.

B—Patient *O*. Diagnosis: extrauterine pregnancy, fourth day after operation.

The broken line is the average for normal blood.

TABLE 2

Time, (min)	2	2·5	3	3·5	4	4·5	5	5·5	6	6·5	7	7·5	Total
H %	1·1	3·8	15·2	22·1	18·3	13·8	10·4	6·9	4·2	2·5	1·2	0·5	100·0
	2·2	9·5	45·6	77·3	73·2	62·1	52·0	38·0	25·2	16·3	8·4	3·7	413·5

for patient *O*, i.e. the number of red cells of heightened stability is increased. The stability index is 1·14.

The curve for patient *B* is displaced to the left, i.e. red cells of reduced stability predominate. The stability index is 0·72.

It should be remarked particularly that a series of erythrograms makes it possible to follow the dynamics of qualitative changes in red cells. Fig. 6 shows a series for a rabbit poisoned with phenylhydrazine. The curves show that in the first days after poisoning the stability of a large fraction of the erythrocytes falls (rise in the left-hand branch of the curve). This period corresponds to the development of marked haemolytic effects.

In the next stage, when regeneration begins, the curve alters quite differently: the right-hand wing of the curve rises and spreads out, the number of red cells with raised stability being increased, and cells of stability higher than that found in the normal even occurring. The number of red cells of raised stability is sometimes so high

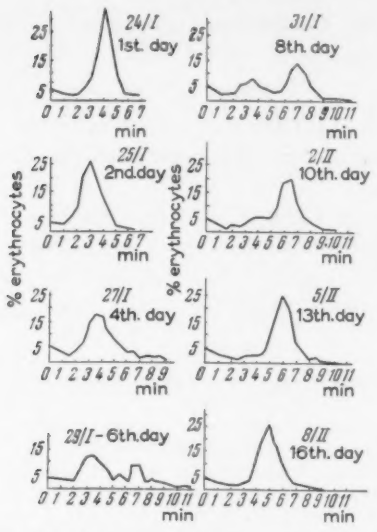

FIG. 6. Changes of erythrogram during acute phenylhydrazine poisoning.

that a second maximum occurs (Fig. 6, sixth to seventh day). The stability indices in this experiment, calculated relative to the initial erythrogram, are as follows:

TABLE 3

Days	1	2	4	6	8	10	13	16
Indices	1·00	0·78	1·05	1·25	1·54	1·52	1·56	1·35

During the haemolytic initial period the index was less than 1, during the regeneration phase it was greater than 1 and an appreciable drop occurred only on the sixteenth day.

This example is given in order to show that the stability distribution of the erythrocytes is a reflection of their physiological state, which leads one to conclude that the method is suitable for wide use in experiments and clinical studies.

The erythrogram method may be recommended for the qualitative study of red-cell composition, in view of the growing need for differential methods of studying red cells, and the difficulty of doing this by a morphological method.

An erythrogram can be obtained with any haemolytic agent. We have studied thermal, photodynamic, alkaline, acid, saponin and osmotic haemolysis. Detailed description of these lies outside the scope of this paper. Of the various forms the most convenient method is a chemical one, a strong inorganic acid or a buffer mixture of the same pH being used as the agent.

That a chemical method is recommended does not rule out the possibility of using osmotic or other forms of haemolysis, since in some cases they are more sensitive to changes in the state of the erythrocyte than is acid haemolysis.

In conclusion, we should state that erythrograms can be taken with equipment

simpler than our relatively complicated apparatus: in particular, a photoelectric erythrometer specially designed for clinical red-cell analysis can be used.

CONCLUSIONS

(1) A differential method for the determination of the qualitative red-cell composition of blood is proposed, based on photoelectric measurement of acid haemolysis kinetics.

(2) The erythrograms give the percentage distribution of erythrocyte stability.

(3) They also exhibit the relation between the stability of the erythrocytes and their condition, and can be used to follow dynamic changes of red-cell composition in physiological and pathological conditions.

Translated by J. E. S. BRADLEY

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A TECHNIQUE FOR ELECTROGRAPHIC REGISTRATION OF DIURESIS IN CHRONIC EXPERIMENTS

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(*Received* 20 *June* 1956)

THE classical method of recording urine output suggested by I. P. Pavlov and L. A. Orbeli results in loss of function of the bladder and of the urinary tract, which makes this method of little use for certain types of experiments. With this in mind, we tried to work out a method allowing the study of diuresis in each kidney, without destroying the mutual relationships of the excretory organs. This we managed to achieve by the recording of biopotentials in the ureters.

The literature available [1–4] contains some scrappy information concerning the link between the functions of the ureters and their bioelectrical activity. These data, based on acute experiments, show, for example, a change of potential in the ureters at the point where they are distended [3], and the action on them of other factors causing contraction of their smooth muscle. Only Bross [4] noticed the increased negativity of the basal potential of these organs when urine output increased.

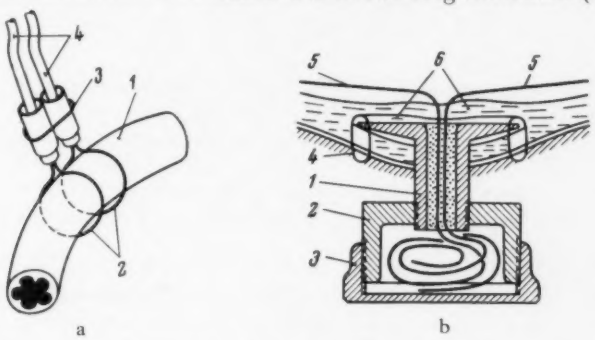
We have met with no other work on the study of the biopotentials of the ureters, or, to be more precise, of their musculature in acute or even in chronic experiments.

Our method of obtaining an electroureterogram (EUG) consists of leading off potentials by means of electrodes implanted on to the surface of each ureter. The electrodes consist of lengths (15–20 mm) of silver wire of diameter 0·3–0·4 mm. They are soldered to the ends of fairly long multicore flexible leads with chlorvinyl insulation.*

The completed single electrodes are tied in pairs with silk thread, and a definite inter-electrode distance is established, equal to 7–8 mm. If greater inter-electrode distances are employed the electroureterogram is masked by potentials from the heart and nearby vessels, organs, etc.

The actual operation for implanting the electrodes is not complicated: in a dog under morphine-ether-chloroform narcosis the abdominal cavity is opened along the mid-line from the umbilicus to the pubic symphysis and the ureter is prepared for a distance of 8–10 mm. The U-shaped electrodes are placed under it, and their free ends are twisted to the corresponding leads.

In this manner the ureter is contained within two ring-electrodes (Fig. 1a).



a b

FIG. 1a. Method of application of the electrodes to the ureter: 1, ureter; 2, electrodes; 3, silk joining the separate electrodes into a pair; 4, leads.

1b. Longitudinal section of the hollow fistula for the leads from the ureter electrodes: 1, fistula tube; 2, head; 3, cover; 4, wire clips holding the tube firm; 5, leads; 6, connective tissue layer, which forms soon after putting the fistula in place.

The second pair of electrodes are implanted anywhere on the other ureter in a similar manner.

The leads are "hemmed" to the abdominal wall, but between the first "hem" and the electrodes a small loop of flex is left, which ensures protection against movement of the animal or excursions of the organs. Then the leads pass to the exterior through an abdominal fistula, the canal of which is filled with wax (with the aim of preventing entry of infection).

The fistula (Fig. 1b) is made of Plexiglas. It is in sections and consists of a tube, a head and a cover, joined together by a screw thread.

The method of installing such a fistula is as follows: the tube of the fistula, with the wires passing through it, is placed through an aperture through the abdominal wall at one side of the original incision. On to the tube is screwed the head, in which are placed the free ends of the leads, and the fistula is closed by a metal cover. It is

* It is possible to replace these leads by silk insulated ones; however, after preparing a pair of electrodes they must be treated with polystyrene, after which the electrodes themselves must be cleaned.

desirable to fix the fistula with nickel or other inoxidizable wire, passing it as shown in Fig. 1b through the abdominal wall and the rim of the opening of the fistula. The animal needs no special post-operative care.

For setting up the experiment the animal is kept in a screened cage. The biopotentials are fed to the input of an amplifier with a balanced circuit and are recorded on a moving strip or are seen on a cathode ray oscillograph. The tracings shown can

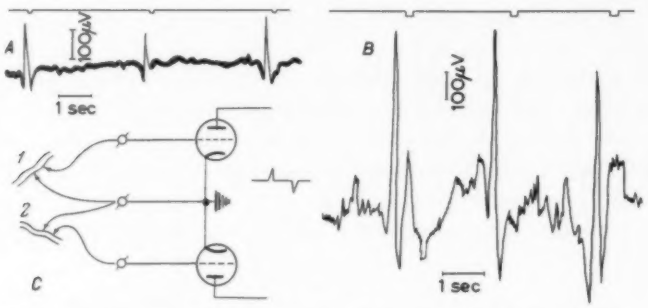


FIG. 2a. Electroureterogram (EUG) of the right ureter (chronic experiment). Above, record of each drop of urine (0·03 ml) leaving the ureter.

2b. Electroureterogram of the right ureter (acute experiment). Each portion of urine is 0·2 ml.

2c. Method for simultaneous recording of the potentials of both ureters by one amplifier: 1 and 2, ureters. On the right, tracing obtained by this method.

be taken as typical of such records. In Fig. 2a is shown an EUG obtained in an experiment on a dog, where besides the recording of biopotentials each drop of urine passed out of the end of an exteriorized ureter was recorded automatically. On the basis of the data obtained, the conclusion was drawn that the rapid changes of potential of a one- to three-phase character—also seen in the EUG by other authors [2, 3]—result from peristaltic contractions of the ureter musculature, by which the successive portions of urine pass to the bladder. We were also able to establish that in a chronic experiment each such increase in amplitude of 200–250 μV at a frequency of 5–6 c/s corresponds to 0·02–0·3 ml of urine.

With increased amplitude and, especially, increased period of oscillation of the peaks, the quantity of urine in one portion is increased. This last relationship has only been obtained so far in acute experiments (Fig. 2b).

The property of a push-pull amplifier of amplifying heterophasic signals can also be used in this work. In such a case, the method of recording is slightly altered. Tracings obtained in this way are shown in Fig. 2c. The difference between this and the previous tracings is that the potential changes from the left ureter, resulting from its peristalsis, are measured by an upward deviation and those from the right ureter by a downward deviation from the isoelectric line.

Using the technique described, it was also possible to obtain fairly accurate representation of the diuresis, by tracing the biopotentials by an electromagnetic stylus on a kymograph; for this it is necessary to replace the voltage amplifier by a more powerful amplifier (for example, an ordinary cine-amplifier).

The technique suggested may in our view be applied as a whole to the carrying

out of experimental work where it is sufficient to have an indirect indicator of urine output at any moment of the experiment, and also in work involving the simultaneous recording of many functions in a chronic experiment.

Translated by J. J. OLIVER

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CURRENT EVENTS

THE ELECTROPHYSIOLOGY AND BIOPHYSICS OF THE SENSE ORGANS AT THE TWENTIETH INTERNATIONAL PHYSIOLOGICAL CONGRESS

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THE Twentieth International Physiological Congress was held in Brussels from 30 July to 4 August 1956. About three thousand delegates participated in the work of the Congress, and in 54 sections and 13 plenary sessions, heard 959 communications on the most varied physiological questions.

The organization of the work of a congress at which it was possible not only to hear, but also to discuss, such a quantity of work is of some interest in itself. The work was conducted in the following manner: during the first half of the day 16 sections worked simultaneously, whilst in the second half 3–4 plenary sessions—symposia—were held. At the sectional meetings papers were read on the results of experimental work, whilst at the plenary sessions summary lectures were given on specific problems. In the section 15 min were devoted to each report, including discussion, and in the symposium 45 min. At his own discretion, each member might reserve time for discussion, but only within the limits of the period assigned to him: not a single minute was allowed in excess of the time shown in the programme. Therefore, as a rule, communications to sections lasted 7–8 min, being followed by a short and very concrete discussion. To save time, those taking part in the discussion usually spoke from their places. In addition to communications, films were shown each morning and demonstrations were made of the latest apparatus in action.

Scientists from all over the world took part in the Congress. Each delegate wore a badge indicating his surname and the country he represented. This was a great help in enabling delegates to meet one another. Our badge, bearing the inscription "U.S.S.R.", assured Soviet delegates a cordial reception from the scientists of most countries. To give the reader some idea of the range of fundamental problems dealt with by the Congress, we now cite the list of questions discussed in the symposia: (1) structure and functioning of the brain; (2) excitation and inhibition; (3) origin of sensory impulses; (4) physiology of the reticular formations; (5) physiology of acceleration; (6) physiology of the heart; (7) thirst and appetite; (8) physiology of the stomach; (9) muscle physiology; (10) hypothermia; (11) drug antagonism; (12) psychophysiology; (13) the pharmacology of the hypothalamus.

Twenty per cent of the total number of the communications were devoted to the

physiology, biophysics and biochemistry of the nervous system. Over one third of these communications dealt with the sense organs.

In the communications on the physiology of the nervous system and the sense organs, there was a predominance of investigations on individual nervous elements of both the peripheral and the central nervous systems. This work was carried out with the help of the microelectrode technique, brought to a high degree of perfection. Microelectrodes of a calibre of 0·3–0·5 μ, and in some cases of less than 0·2 μ, were introduced either into the body of the nerve-cell or into its axon and the physiologists studied the responses not only of individual nervous elements, but also of the separate parts of these elements. On the basis of the characteristics of the electrical responses, very extensive physiological generalizations were made, some of which possessed novelty and interest.

Of 60 communications on the sense organs, the greatest number dealt with the physiology of vision, and 15 with the physiology of hearing and with other forms of reception (taste, smell, cutaneous reception).

To enable the reader to form an idea of the whole scope of the Congress's work on the sense organs, we shall also give a short account of the contents of those communications that have already been published abroad.

Cutaneous reception: The sectional meeting entitled "Taste, Hearing" commenced work with three communications dealing with cutaneous reception; among them was the very interesting investigation of H. Hensel (Federal Republic of Germany) on the action potentials in response to various temperature stimuli of an isolated cutaneous receptor of the shark (ampulla Lorentsini). Hensel discovered that this receptor responds sharply to temperature stimuli by variation of electrical activity. At a constant temperature the individual fibre gives a prolonged discharge, the impulse frequency reaching a maximum (65 impulses/sec) in the temperature range of 15–23°. At higher and lower temperatures, the impulse frequency falls, reaching zero at a temperature of under 2° and above 34°.

The high sensitivity of the electrical activity of the ampulla to variations of temperature was established—with an increase of 0·1°, the number of impulses increased by 7, and with a reduction of 0·1° the number changed by 20 impulses/sec. The application of thermal influences to an active region of an individual fibre of the ampulla brought the impulse frequency to 180 per second. Shifting of the point of temperature variation by 0·1° produces a still more significant change in the impulse frequency (to 90/sec). This work has shown that the thermoelectric reaction of the individual cutaneous receptor of the shark corresponds qualitatively in all respects to the behaviour of the thermoreceptors in warm-blooded creatures, while from the quantitative point of view it is even more sensitive.

J. Hardy and E. Hendler (U.S.A.) reported on radiometric measurements of the temperature of the human skin in relation to temperature sensations. In these experiments a new type of radiometer was used by means of which the temperature of the skin was measured with an accuracy of ±0·0001° over a period of 20 min. At the same time the subject gave a verbal account. It was shown in the report that the temperature of the skin varies widely, changing up to 0·2° in 5–10 sec, during which no temperature sensations occur. However, a continuous change in the level of the skin temperature, even in the range ±0·0001° per sec, causes a temperature sensation.

It is interesting that the verbal indication that it will be warm is accompanied by a rise in the temperature level of the skin, and the suggestion of cold by a fall.

In the work of P. Stern and S. Hukovic (Yugoslavia) the dependence of sensations of pain on the histamine content of the skin was shown.

Thus these investigations on cutaneous reception have disclosed two important facts: (1) the extreme sensitivity to temperature variations in man and (2) the presence in fishes of a very high reactivity to temperature variations. Such a high reactivity may constitute a kind of "direction finder", enabling fishes to find their way to the location of their food bases.

The physiology of taste: The following three communications to the section "Taste, Hearing" were concerned with the electrophysiology and biochemistry of the gustatory organ. One of them is of fundamental importance for the physiology of the sense organs in general.

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The communication was made by M. Cohen, S. Landgren, L. Storm, and Y. Zotterman (Sweden) on the question of cortical responses to electrical and natural stimulation of the tongue in cats. The potentials were recorded by the aid of microelectrodes buried at steps of 0·2 mm to a depth of 3 mm below the surface of the cerebral cortex. The flow of afferent impulses along the chorda tympani was recorded from the region of the middle ear. Groups of fibres with different thresholds and rates of conduction were found.

By suitably selecting the intensities of stimulation, it was possible to correlate the cortical potentials with the responses of these groups of afferent elements. The conclusion was reached that the most important surface-positive phase of the cortical potential elicited arises not in the gustatory, but in the tactile, cells. This potential was used in composing a map of cortical gustatory projections. The record was taken extracellularly from approximately 50 tactile cells. These, for the most part, were distributed at a depth of about 1 mm and never responded to gustatory stimulation. A great number of tactile cells could be activated by electric stimulation of the trigeminal part of the lingual nerve, as well as of the chorda tympani. These cells give a characteristic picture of activity with a short initial outburst which, after an interval lasting 50 msec, is succeeded by a protracted continuous discharge. This is the typical picture of electric activity for the majority of cortical tactile cells.

Meanwhile the authors of this investigation discovered only five cells of the gustatory zone of the cortex responding to gustatory stimuli; they are smaller than the tactile cells, are distributed at a considerable depth (2 mm) in the cortex, and respond to stimulation by salt, acid, bitter and water, but they do not respond to touch. Along with them there are tactile cells. One cell was discovered which specifically responded to cooling of the tongue, but not to warming or to gustatory stimuli.

Y. Zotterman (Sweden) with the help of microelectrodes, studied the presence of "aqueous" taste in various mammals. He showed that stimulation by water elicits an electric response in cats, dogs, pigs and rabbits; rats possess no fibres susceptible of stimulation by water. The author analyses the question of special "aqueous" receptors in various mammals.

The third communication in this section was made by G. Bourne and A. Baradi (England) on the histochemical localization of enzymes in the gustatory and olfactory organs.

The electrophysiological studies on the gustatory organ submitted to the section, make it possible to regard as established in principle the important fact of the presence in the cerebral cortext of nervous elements selectively reacting to the quality of a specific stimulus—to bitter, acid, salt or temperature. Furthermore the presence of a special "aqueous" receptor in the majority of mammals has been revealed.

Thus it has been found that the mammal distinguishes a much larger spectrum of gustatory stimuli than was previously supposed, and that the spectrum selectively stimulates cortical cells of the gustatory zone of the cerebral cortex.

The electrophysiology and biophysics of the organ of hearing: The second half of the meeting of the section "Taste, Hearing" was devoted to the physiology of the sound analyser. In a large number of these communications electrophysiological methods of investigation were used, making it possible to discover several aspects of the working of the organ of hearing.

One of the most interesting studies was presented by R. Galambos (U.S.A.) on the "Physiological investigation of the descending auditory tracts". With the help of physiological experiments, the author studied the system and functional peculiarities of the descending fibres in the auditory analyser of the cat, which have so far been studied only anatomically. He showed that stimulation by electric impulses of the fibres of the olivocochlear fasciculus connecting the superior olive with the contralateral cochlea, caused a reduction in the afferent impulses, arising at the periphery under stimulation by sounds.

The second series of experiments was carried out with chronically implanted electrodes on animals under conditions of unrestrained behaviour. The potentials, provoked by an auditory stimulus, were recorded in the cochlear nucleus, the medial geniculate body and the auditory cortex. The results of Galambo's experiments showed that the electrical response is negligible or absent in an animal exposed simultaneously to a visual stimulus, of medium intensity, in an animal adapted to the application of a sound, and great when the sound is a signal of painful stimulation.

Y. Katsuki, T. Sumi, and K. Uchiyama (Japan) reported several characteristics of the electrical responses from auditory neurons in cats under auditory stimuli. The potentials were detected with the help of superfine microelectrodes (pipette diameter less than 0·2 μ, filled with 3 m KCl). Electrical activity was recorded in the cochlear nerve tract, the cochlear nucleus, the trapezoid body, the inferior corpus quadrigeminum, the medial gericulate body and the cerebral cortex.

They studied the responses of individual elements, obtained either from the axons (mostly), or from the body of the neurons. The latter were distinguished by slow graded potentials and large resting potentials exceeding 50 mv. All the spikes were positive and mono- or di-phasic, from 1 to 5 mv. Spontaneous discharges were frequently observed in the peripheral, and much less frequently in the central, parts.

The peripheral neurons responded to strong auditory stimuli over a wide range of frequencies, but the central neurons only to a much narrower range of stimulus. The zones of the responses were determined by stimulation by long and short pure tones. In the upper projections, adaptation to sound arose considerably more quickly: the majority of elements responded only at the beginning of the stimulus. Elements inhibited by stimulation, and pure "off"-elements, were detected. Within the cells,

both positive and inhibitory changes of the potentials were discovered, similar to those also observed in other synapses.

The authors showed the interaction of the electric responses of two stimuli even in the peripheral regions of the auditory analyser—the response to one sound was inhibited by another sound.

N. Kiang and M. Goldstein (U.S.A.) studied the response of the auditory cortex to brief tones and noises. Blunt electrodes were placed in the "low-frequency points" I and II of the zone of the auditory cortex of an anaesthetized cat. The thresholds of the electrical responses were determined for pure tones and for short noise signals. The curves of the dependence of response-frequency on threshold intensity were studied. The results of the experiments were analysed from the point of view of spatial distribution and frequency-sequence, as well as from that of the more general mechanisms of sound perception: according to this point of view, the acoustic stimulus undergoes a specific spectral decomposition at the cochlear level, whereas the frequency-sequence stimulates directly, and gives rise to a nervous response following with the same, or some other, frequency. The mechanism of spatial distribution serves for the analysis of high-frequency sounds, whilst the peripheral nervous response participates directly in the analysis of low frequencies, just as in pure low sounds, the frequency of the peripheral nervous impulses follows the frequency of the sounds.

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J. Hughes (U.S.A.) showed that after repeated stimulation by sound, an enhanced reactivity of the auditory system occurs, continuing for approximately 2 min after the interruption of the application of the sound. This was demonstrated experimentally: in man by determination of the threshold of audibility and electrophysiologically, in the cat by leading off the potentials from the fenestra rotunda; in the latter case undulatory oscillation in the amplitude of the electric response was observed.

Thus, in these communications on the variations and characteristics of the electrical activity of individual elements and separate links of the sound analyser, the mechanism of the analysis of sound stimuli was studied. On the parameters of the electrical activity the following facts were established experimentally: (1) in the cases where the auditory stimulus acquires a signal significance, an intensification in the electrical activity of the various sections of the auditory system takes place; (2) the processes developing in the descending auditory neurons can regulate the magnitude of the signal passing along the classical afferent tract; (3) the interaction was shown of the electrical responses to two auditory stimuli even in the peripheral regions of the auditory analyser, whilst for sound and light stimuli this interaction was established at higher levels of the auditory analyser; the electrical response to one signal is inhibited by the other signal; (4) it was shown that in proportion to the transition to higher levels of the auditory analyser, the range of frequencies at which the electrical response of the neuron arises narrows; (5) there was discovered a sensitization to sound lasting about 2 min after repeated auditory stimuli.

Some of the facts enumerated were known previously in physiological acoustics, but they were obtained mainly by determining the thresholds of audibility in experiments on man; now variations in electrical activity provide corroboration of these facts.

Several communications were devoted to a thorough study of the nature of this electrical activity.

In Katzuki's communication, considerable attention was given to the characteristics of the electrical responses of individual neurons, and separate parts of these neurons, to auditory stimuli. The virtuosity of these masters of microelectrode technique enabled them to study with precision the responses of the separate parts of the neurons.

A communication by C. Euler, H. Magoun, and G. Ricci (U.S.A.) was made on this same subject. It dealt with the characteristics of the potentials arising in the auditory cortex from electrical stimulation of the medial geniculate body of the non-anaesthetized cat. The response to a single shock consists of an initial deflection of short duration of a surface-positive phase and a subsequent negative phase. The surface-positive wave has a smaller threshold than the subsequent negative component. These two components were obtained separately from different points of the auditory cortex. Distortion of the polarity of both components of the response recorded by penetrating electrodes was obtained at approximately one and the same level. The two components of the cortical response to stimulation of the medial geniculate body interacted differently with responses to other stimuli.

The results of the experiments testify to the fact that the surface-negative phase is bound up in its origin with spread of excitation to neurons localized in the surface of the cortex, but not to spread on the surface along the apical dendrites of the neurons responsible for the preceding surface-positive phase.

L. Frishkopf (U.S.A.) carried out the correlated analysis of the cerebral "resting" potentials and auditory stimulation in man. In a soundproof chamber the resting currents were studied, after which sounds of varying duration and intensity were applied. For the analysis of the electroencephalogram, obtained from the temporal and occipital region, three methods were used: (1) the autocorrelation of activity obtained from specific regions of the cranium; (2) the cross-correlation of activity obtained simultaneously from two regions; and (3) the cross-correlation of activity observed with short rectangular pulsations.

One communication was made to the section on the physiology of hearing dealing with cybernetics. It was entitled: "A probability approach to the study of the electrical activity of the auditory nervous system." The author of this communication (W. Rosenblith (U.S.A.)), submitted that a mathematical model can be employed in the study of the responses obtained by blunt electrodes from the various structures and points of the auditory system. Starting from data on the relative frequencies of the potentials arising in a single element in response to various intensities of stimulus, and from variations of the threshold, a number of statistical parameters of the mathematical model can be evaluated, for responses of the auditory nerve and the cortex to auditory stimulation. The effect which the activity of a given nervous element produces on an adjacent element was computed. The author considers that much might be elucidated for the prediction of neuron behaviour by using calculating machines.

Y. Kuiper (Holland) examined the relation between the amplitude of the stimulus and the intensity of the microphonic effect, originating from the current circulating around the cells of the labyrinth. The results of his experiments support the assumption that acetylcholine is involved in the microphonic effect.

Physiology of vision: The three sectional meetings devoted to the physiology of vision were entitled: (1) Photosensitivity, (2) The eye—the visual cortex, and (3) The physiology of the eye. The meeting of these three sections was begun with a series of interesting communications on the photochemistry of visual pigments, and was followed by communications on the physiology of the retina, and later, on the physiology of the higher regions of the visual analyser.

Electrophysiology of the retina: In the sections on the physiology of vision, an extensive number of studies of the potentials of individual elements, and on the total electrical activity of the retina, was submitted.

A lively discussion developed in these sections as to the site of the generation of electrical activity in the retina. The discussion arose from the presentation of a series of experimental works, which had led the authors to conflicting conclusions.

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Y. Gonella, L. Cornu, and P. Benoit (France) analysed the retinogram of the frog by means of the simultaneous recording of the ordinary retinogram, and a so-called "internal" retinogram, i.e., a recording of the intra-retinal negative wave obtained with the help of microelectrodes. The experiments were conducted on the open enucleated eye of the frog after it had been treated with various poisons. Variation in the form of the electroretinogram proportionate to its activation by strychnine led the authors to suggest that waves *b* and *d* of the electroretinogram are an expression of the negative intra-retinal wave. This suggestion was confirmed by subsequent experiments, in which the application of a current of suitable direction and intensity (strength of current at the internal anode above 100 μA) reversibly neutralized the negative wave of the internal retinogram and preserved the usual form of the total electroretinogram. The results of these experiments led the authors to conclude that the genesis of the electrical activity is dependent on many sources, and to refute decisively Svaetichin's hypothesis, held up to now, to the effect that the electroretinogram is produced by the photoreceptors only.

P. Denis (Belgium) reported on the action of various pharmacodynamic agents on the electroretinogram of the rat. His experiments support the contention that the retinogram has its origin in biochemical processes, occurring in the retina.

At the same time G. Svaetichin, H. Fernandez-Moran, and R. Jonasson (Venezuela) communicated the results of an electrophysiological and electromicroscopic study of the compound eye of insects. With the help of an extremely fine technique, they made a detailed study of the nature and place of origin of electrical activity in the compound eye of insects, and reached the following conclusions: (1) the action potential is generated in the basal part of the photoreceptors, (2) the resting potential is connected with the basal part of the retina. It is interesting that, according to the data advanced by these authors, the structure type of the lamellar discs in the photoreceptors is similar to that in the photoreceptors of warm-blooded creatures.

In the section on the physiology of vision, many communications dealt with the characteristics of the electrical activity of the retina in relation to the various functions of vision.

Several communications were made on the spectral sensitivity of the retina. K. Motokawa (Japan) reported his work on the reactivity of individual elements of the retina to monochromatic radiations. The potentials were recorded by microelectrodes of a calibre of 0·3–0·8 μ from the retina of the carp with the help of a direct

current amplifier. According to the author's data, the resting potential was equal to 30–50 mV; on exposure of the eye to light a receptor potential (action current) equal to 5–10 mV appears, which accords with Svaetichin's data. However, in many cases this potential had another sign, even at a magnitude of 10–30 mV. It is a highly important fact that the magnitude of the receptor potential sometimes changed in inverse relation to the wavelength of the stimulating light. Curves of spectral responses of a single element of the retina were constructed: (1) on the magnitude of receptor potentials in response to stimulation by spectral illumination of a given wavelength (λ) of the equal-energy spectrum; (2) on the measurement of spectral sensitivity in graded changes of 10 mμ. In both cases four forms of receptor with maximum responses at λ equal to 650, 600, 550, and 470 mμ were revealed. Each response curve had a complex form—one fundamental and three secondary maxima. The latter coincided with the calculated points of the spectrum.

On the basis of his experiments, Motokawa comes to the conclusion that the receptor cell contains four kinds of photosensitive substances, and is characterized by the substance of the highest concentration.

J. Armington and W. Biersdorf (U.S.A.) studied in two subjects the influence of chromatic adaptation on the human electroretinogram. The latter was recorded with the help of electrodes mounted in a contact lense. The relative spectral sensitivity was determined by the help of a set of interference filters for each state of adaptation at different levels of brightness.

If the electroretinogram was produced by small (20 flashes/sec) monochromatic stimuli superimposed on the constant adaptation-fields, no chromatic effects were observed. The curve of spectral sensitivity was in all cases similar to the curve of visibility of the photopic eye, irrespective of the colour of the adaptation field. When the brightness of the adaptation field was increased, the spectral sensitivity declined uniformly throughout the whole spectrum.

Whenever the adaptation field disappeared at the moment of the presentation of a prolonged test stimulus, an effect of chromatic adaptation was observed, resulting in a considerable and specific depression of the spectral sensitivity. The curves of spectral sensitivity gave three clearly-expressed maxima at wavelengths of approximately 500, 550, and 620 mμ, at which the corresponding chromatic adaptation produced a lowering in the corresponding maximum. However, neither of the techniques of investigation made it possible to separate the corresponding components at short wavelengths.

These studies are fundamentally important for the physiology of vision, so that if they are correct, the prevailing concepts of the characteristics of the reaction of the retina to light must be revised.

The interesting question of the relation between variations in the electroretinogram of man and his perception of colour stimulation at varied brightness was also considered.

H. Ascher (England) reported his work on the study of the human electroretinogram under the influence of different levels of brightness. A contact lens with a silver lamina served as an electrode. A flash of light, uniformly illuminating the whole field of vision, lasted 0·05 sec. At low brightness, the amplitude of the positive wave *b* was proportional to the logarithm of the intensity of the light flash, but with

an intensity of light above $10^{2 \cdot 5}\times$ the threshold of the retinogram, the amplitude of wave *b* remained unchanged.

It is noteworthy that the flash was perceived by the subject at intensities less than that required for the appearance of the retinogram, whilst sensation of increasing brightness corresponded with the raising of the intensity of the light, right up to the highest levels ($10^{5 \cdot 5}\times$ threshold of the retinogram).

Thus, no correspondence was established between the perception of brightness and variation of wave *b* in the electroretinogram. At the same time wave *a* was observed at every brightness used in the experiment, and increased in amplitude even at the highest intensities of light.

Ascher's work bears testimony to the fact that the perception of brightness corresponds more closely to wave *a* of the human retinogram than to wave *b*.

The sensitivity of different zones of the retina to white light was studied from the electrical responses of individual elements of the retina. H. Wagner and W. Wolbarsht (U.S.A.), recording the electrical impulses coming from individual axons of the ganglion cells of the retina, constructed a map of points possessing identical sensitivity to a white patch of 0·05 mm dimension. Regions with a maximal sensitivity and with a gradual diminution of this in proportion to the distance from the centre were made out. The spatial summation of two equal light points proved effective up to 1 mm. The temporal summation went in a straight line for time intervals lasting up to 0·2 sec and was less marked for a longer period.

Papers were presented to the section on the physiology of vision, in which the authors compared contemporary data on the electrical reaction of individual nervous elements of the visual analyser and on the different functions of vision.

Thus M. Pirenne (England) invoked the functional characteristics of the *off*-elements of the retina for the analysis of the mechanism of visual acuity. He suggests that thanks to the graduated movements of the eye in a fixation pause, the image of a black object falls on that part of the retina, which had so far been exposed to background illumination, and this may lead to stimulation of the *off*-elements which react, as is well known, to electrical activity at the shutting-off of the illumination.

This hypothesis, in our view, does not seem very convincing and is unlikely to find any wide acceptance.

C. Haig (U.S.A.) attempted to apply the data obtained by Hartline to the receptor fields of the human retina, and came to the conclusion that summation phenomena of photoreceptor elements united by a single ganglion cell were observed in man in every part of the retina examined. Haig confirmed Ricco's law for small areas, explaining its inapplicability to large areas by variation in the density of the photoreceptor elements on the surface. A comparison of this kind is permissible, but hardly new: it had already been made some time ago by other research workers, particularly by the author of the present article in 1948.

The electrophysiology of the central parts of the visual analyser: One of the most interesting problems broached in the section on the physiology of vision, was the question of the specificity of the reaction of the nervous elements of the brain to visual stimulation. Until recently, as is well known, it has not been clear whether the cells of the central parts of the visual analyser react only to the strength and

duration of the visual stimulus, or whether they also respond selectively to specific properties of the light (for example, to its colour saturation).

Several papers were read on this subject. Margarita Lennox (Denmark) studied the responses of individual elements of the upper third of the optic tract to stimulation by flashes of light of various wavelengths, durations, intensity, and frequency, and also to electrical stimulation of the optic nerve. The responses of single elements, recorded with the help of microelectrodes of a calibre less than 0·5 μ, were compared with the total electrical response from this part of the visual analyser, established with the help of a blunt electrode of 0·3 mm.

Lennox discovered a selective reactivity of single elements of the central parts of the visual analyser to the colour of the stimulus. In the majority of elements, on stimulation with blue light, the threshold was lower, the frequency of the spikes of the *on*-elements was higher, while the latent period was longer than in stimulation by red light.

Lennox also showed that the single elements of the upper third of the optic tract responded to light, either by a discharge of impulses or by a suspension of activity and the generation of a discharge in the dark, i.e. they responded in the same way as single elements of the retina and single fibres of the optic nerve. However, in contrast to Granit's data, only a minute part of the elements responded with an *on-off* effect, though pure *on*- and pure *off*-elements were encountered equally often, the type of response of each element being constant and not dependent on the parameters of the stimulus.

D. Ingvar (Sweden) also elucidated, from the change of the electrical activity, how the discrimination of wavelength as well as of brightness is brought about in the central visual tracts. She demonstrated the increase in sensitivity of these tracts to the blue and green parts of the spectrum, observed at certain levels of intensity of stimulation. Ingvar conducted experiments on the non-anaesthetized "isolated brain" of the cat, studying simultaneously the spectral sensitivity of the visual cortex contralateral to the stimulated eye, and of the corpus quadrigeminum, the external geniculate body, the brain stem and the retina. This sensitivity was determined by studying electroretinograms and by measuring the magnitude of the latent periods of the cerebral potentials, obtained by means of blunt electrodes.

Ingvar also demonstrated that the curves of the spectral sensitivity of the retina frequently coincided with the curve of the visual purple, though at times their maximum appeared displaced towards the blue end of the spectrum.

Thus present-day experimental data obtained by the electrophysiological method make it possible to regard the question of the presence of a selective reaction to specific stimuli in the upper parts of the visual analyser as answered in the positive.

No less new is the attempt to follow the reflection of trace effects in the change of electrical activity in the upper third of the visual analyser, carried out by Y. Hughes (U.S.A.) in his work on the postethenic electrical activity in the auditory and visual systems. On stimulation of the optic nerve of the cat, potentials in the geniculate body and visual cortex were recorded. The author discovered that after repeated stimulation an undulatory oscillation of the amplitude of the potential arises—at the beginning sub-normal, then above normal, and afterwards again sub-normal, lasting up to 7 min. This reaction took place in the upper part of the visual tract. It is

interesting because of its duration, which corresponds to the after image and may be connected with psychical reactions.

The question of the reaction of the elements of the visual cortex to intermittent chromatic stimulation was also considered in the section on the physiology of vision.

O. Grüsser and O. Creutzefeldt (Federal Republic of Germany) studied the frequency of fusion of flashes in the reaction of single neurons, and in the total response of the optic cortex, in the "isolated" cerebrum of the cat. Flashing light of varying frequency served as the stimulus. The authors of this work established that the fusion frequency of the flashes recorded by the reaction of individual neurons increases with the intensity of illumination (at 30 lux it is equal to $12{\cdot}5 \pm 2$, at 500 lux to $18 \pm 1{\cdot}5$). The latent period of the reaction of the *B*-neurons (corresponding to the retinal *on*-elements) increases together with the frequency of the flashes right up to fusion; it remains unchanged, or falls, in the *D*-neurons (*off*-elements) and shows varying changes in the *E*-neurons (*on*- and *off*-elements).

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The primary surface-positive potentials in the electroencephalogram fuse at those frequencies of flutter which correspond to the maximum frequency of fusion in individual neurons, whilst the fusion of the secondary waves (surface-negative) takes place, according to the intensity of the stimulus, at 5–12 flashes/sec.

In Creutzefeldt's work this same question is subjected to a still more profound and exact study; in particular, the dependence of the reaction of single neurons and of the electroencephelograms on the adequacy or inadequacy (for the visual analyser) of the stimulus was considered.

The work of M. Van Hoff (Holland) dealt with the question of the reaction of the visual area of the cortex to intermittent visual stimulation of the retina in man and cat. The author of this study showed that below a definite limit of the duration of the light flash, the *off*-effect is absent, whilst the *on*-effect does not set in until the length of interval between the flashes has reached a critical magnitude.

In these investigations it was shown that variation of the frequency and duration of the light flashes is reflected in the changed electrical activity of the individual elements, and in the total response of the visual cortex and the subcortex.

Of great interest were the works in which the topography of the visual area of the cortex was established by study of the potentials arising in response to visual stimulation of different parts of the retina in individual cortical elements. The exact projection of the retina in the superior corpus quadrigeminum had been shown previously by Apter in the cat and by Cooper in the monkey.

R. Gaze (Sweden) presented a paper to the Congress on the role of the retina in the optic lobe of the frog. The eye of the frog was placed in the centre of a perimeter, in which there moved a neon source of 15′ dimension. With the help of microelectrodes, multi- and single-element responses of the cortex were recorded, by which it was established that the nasal part of the retina is projected to the postero-internal part, the inferior-temporal to the antero-internal, the upper nasal to the postero-external, the superior-temporal to the antero-external part of the optic lobe. The projection of the central part of the retina was not so exactly elucidated.

Unfortunately the author of the present article was not able to acquaint herself with a single one of the very important and interesting papers on the distribution

of auditory and visual impulses to the sensorimotor cortex, published by T. Feng, Y. Liu, and E. Shen (China).

Thus, at present, we may accept as established that the electrical activity of single elements of the cortex and the subcortex, adequately reflects the various reactions of the visual analyser to visual stimulation. This is borne out by the reports we have cited, which have shown the characteristics of the electrical activity in response to spectral irradiation during trace effects, change of frequency of flashing light, and its localization.

On the purely biophysical works carried out with the help of the microelectrode technique, mention must be made of the paper by H. Luttgau and A. Muralt (Switzerland), who measured the precise variations of thresholds, action currents, rate of increase and decrease of the phases of the action currents, and of the refractory period in single nodes of Ranvier of the nervous fibre on stimulation by monochromatic ultraviolet radiation of different wavelengths. The authors think that the lineal decline established by them, in the rate of increase of action currents dependent on the time of exposure, constitutes a measure of the variation of permeability of the active membrane, whilst the rise of the threshold is a measure of the photochemical activity of the radiation.

The question of the correlation between the total electrical activity of the brain and the potentials taken from single nerve elements of the cortex was discussed in the section on physiology of vision.

In the work of Grüsser and Creutzefeldt referred to above, a similar correlation was established for the frequency of the fusion of the primary surface-positive potentials in the electroencephelogram and the fusion-frequency of the potentials in individual neurons.

A series of papers was presented in which the correspondence of the electrical responses of the retina and of the brain to visual stimulation, was considered.

W. Cobb and G. Dawson (England) studied the form and duration of the potentials produced by bright flashes in the occipital region in man. These authors established the fact that the total activity of the occipital region of the cortex sets in approximately 25 msec after a flash of a brightness of 10^6 foot-lamberts, produced in front of the dark-adapted eye. The authors showed by the method of graphic analysis, that the first positive potential lasts 5–8 msec with an amplitude up to 1·5 μV, then comes the negative wave—with a duration of 15–20 msec with an amplitude of 2–5 μV, after which there is a second positive wave—5–8 μV. A simultaneous record of the electroretinogram did not coincide with the three components just enumerated of the electroencephalogram. A fourth component—small oscillations, consisting of 3–5 waves imposed on the second and third components—has a period very close to the small oscillation in the electroretinogram during bright flashes but this sets in 30 msec later.

F. Crescitelli and E. Gardner (U.S.A.), who stimulated the retina of animals with colour stimuli, compared the electroretinogram with the responses from the visual region of the cortex in animals, possessing a retina containing only rods (guinea-pigs), and a retina containing only cones (squirrels). They studied the time relationships between the retinograms and the resulting cortical potentials in correlation with different wavelengths of stimulating light.

The communications bore out the fact that under stimulation by adequate stimuli a certain correspondence is observed between the electrical responses of the retina and the brain. At the same time this correlation apparently does not exist under the influence of non-specific agents.

P. Visser (Holland) studied the variation of the electroretinogram and the electrical activity in the striate region in the "isolated brain" of the cat on injection of NaN_3 in Ringer's solution. The injection of measured doses of this preparation led to a sudden and short-lived increase in the difference of the potentials between the cornea and the posterior part of the eye. With doses greater than 10 mg, wave *b* of the retinogram gradually disappeared, the effect proving to be stable, whilst the potential of wave *c* increased in proportion to the quantity of solution injected. At the same time, the electrical activity of the central parts of the analyser were gradually depressed under the action of NaN_3, whilst wave *b*, lowered by the preparation, was again restored in the course of a minute after the injection of NaN_3. It is noteworthy that the *on*- and *off*-responses of the striate region of the brain, called forth by visual stimuli, retained a considerable amplitude even when the activity of the visual cortex was strongly depressed, and wave *b* had completely disappeared.

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The electrophysiology and the photochemistry of the visual analyser also received attention in the plenary session on the genesis of the sensory impulse, to which R. Granit presented a survey paper on: "The absorption and nervous transmission of the visible spectrum."

The speaker commenced by referring to one of the most important parts of the physiology of vision—the centrifugal regulation of the retina, and the question of the reverse collaterals in the optic nerve, and to his new experimental material showing the presence of excitatory and inhibitory effects in the retinal ganglion cells.

A little later, Granit came to the fundamental part of his paper: the primary mechanism of stimulation, which he considers indisputably to be purely photochemical.

Absorbed light quanta produce chemical changes in the visual pigments, leading in turn to the generation of excitation potentials. The absorption spectra serve as indicators of the chemically active substances, and the spectral analysis as the basis of the chemical analysis, in as much as it can also serve as the basis of the chemical stimulation of the retina.

Granit then compared the data on the spectral sensitivity of the retina, obtained electrophysiologically, with the measurements of the visual pigments, and with data on the different thresholds in man. In particular he referred to his experimental material on dominators and modulators, stressing that in view of the variability of the electrical responses of individual elements, it is essential to calculate the average results of experiments conducted on many fibres.

Granit devoted the basic part of his paper to a detailed exposition of the most recent data on visual pigments—their stability in solution, and to bleaching, methods for determining the purity of a visual purple solution, methods for studying pigments in the complete retina and in individual photoreceptors *in vivo*. He dealt in detail with the explanation of the maxima of the curves of spectral sensitivity of pigments obtained by various methods of investigation.

On the basis of Dartnell's work on the possibility of disintegrating the pigment

with a maximum wavelength of 530 mμ into three pigments with maxima at wavelengths of 533, 510 and 550 mμ; of Arden's work establishing the maximum of absorption in the suspended living receptor at 544 mμ; and from data showing displacement of the maximum in the long-wave part of the spectrum in the case where the absorption of a single photoelement in comparison with the maximum obtained in a solution of the pigment was measured (Arden, Dobrovol'skii, Johnson and Teneli), Granit came to the conclusion that with the methods which until recently existed for the extraction of pigments from the retina, a selection of certain kinds of pigments took place. Granit established the coincidence of certain of the maxima of the electrophysiological curves of spectral sensitivity, of living photoreceptors and modulators.

The speaker then dwelt on the photographic method of determining *in vivo* the visual purple (Campbell and Rushton and Weale), based on the reflection of a pencil of light from the retina; on the question of the density of the pigments in the retina; on the work of Donner and Rushton, showing that the directional effect of Styles and Crawford is to be observed only in the cones, and not in the rods.

Granit sees a corroboration of his data on modulators in the work of Weale, dealing with the absorption of spectral radiations by the visual pigments, and in the work of Lennox and Ingvar cited above. He also dwelt on the data in which pigments with a narrow zone of absorption were demonstrated in the squirrel. Granit then passed on to the question of the decomposition of pigments according to Weale's data.

He devoted the end of his paper to the evidence that the adsorption curves of the visual pigments obtained by the latest methods were close to the curves obtained by electrophysiological means.

A large number of the papers on the electrophysiology of the visual analyser in the section on the physiology of vision presented novelty and interest. All the works were abundantly illustrated by experimental data, which were usually the subject of lively discussion.

The paper by R. Granit, the outstanding figure in the field of research on the electrophysiology of the retina, compared unfavourably with the rest, in that he did not produce his latest experimental material, but limited himself to correlating the data on the electrophysiology and the photochemistry of the eye. Such a correlation is not new: it was made several years ago in the Soviet Union. The vast audience assembled in the symposium was apparently not satisfied with the paper presented by Granit, who laid emphasis on the corroboration of the results of his own experiments on modulators and dominators by contemporary data on the photochemistry of the retina.

In summing up the papers presented to the Congress on the physiology of the sense organs, it should be mentioned that by far the greater part of them were concerned with the electrophysiology of the various analysers.

A very large amount of the work was carried out on individual nervous elements of the different parts of the various analysers. This method of the physiologist of "listening in" to the individual element or even to its separate parts, makes it possible to elucidate much that is new in the reactions of nervous structures to external stimulation.

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Translated by D. MAITLAND MÜLLER.

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1. **PERRY, S. V.,** Relation between chemical and contractile function and skeletal muscle cell structure. Physiol. Rev. **36:** No. 1, 1–76, 1956

The structural and chemical properties of muscle fibres are reviewed in relation to their contractile function. Data obtained by electron and optical microscopy are employed to analyse the transverse-striation structure of myofibrillae, sarcoplasm, sarcolemma and certain other inclusions. Changes in the transverse-striation structure on muscle fibre contraction and relaxation are examined. The properties and roles of the protein components in myofibrillae (myosin, actin, tropomyosin and actomyosin) are reviewed in relation to the contractile function. An extensive bibliography of 372 titles, up to 1955, is given.

2. **MATTHAEI, E.,** and **TIEGS, O.,** The path of the slow contractile wave in arthropod muscle fibre. Phil. Trans. B**238:** No. 656, 349–354, 1955

The authors recorded the slow contraction of a spider's leg muscle by cinemicrography. The spiral propogation of the slow contractile wave in the fibres was demonstrated. It is suggested that the Z-disc ensures that all the myofibrillae of the fibre are involved and synchronized in the contractile process.

3. **ZHUKOV, E. K.,** The viscoelastic properties of a tonic preparation of striated muscle. Fiziol. zh. **42:** No. 1, 95–101, 1956

The viscoelastic properties of tonic and atonic muscles were studied and compared by plotting extension against load. These properties were also studied in isolated tonic and tetanic fibres. It was shown that the tonic fibres are more extensible, and their viscoelastic resistance increases in tonic contraction is less than in tetanic contraction. The comparatively slow fatigue of tonic fibres is demonstrated. In tonic fibres viscosity was more apparent, giving them a plastic consistency.

4. **TASAKI, I.,** New measurements of the capacity and resistance of the myelin sheath and the nodal membrane of isolated frog nerve fibres. Amer. J. Physiol. **181:** 639–650, 1955

The capacity and resistance of the myelin sheath and the membrane in the region of Ranvier's nodes were measured in an isolated frog nerve fibre. The effects of various chemical substances (cocaine, saponin and hypertonic saline) and of temperature on the electrical parameters were studied.

5. **DODT, E.,** Geschwindigkeit der Nervenleitung innerhalb der Netzhaut. Experientia **12:** No. 1, 34, 35, 1956

The impulse conduction velocity in a non-myelinated part of the optic nerve in the retina of a cat was measured with microelectrodes, after antidromic stimulation of the geniculate body. The mean velocity in non-myelinated "fast" fibres was 2·8 m/sec, and in "slow" fibres 1·7 m/sec. The velocities in the corresponding groups of myelinated fibres were 70 m/sec and 23 m/sec.

6. **KONISHI, K.,** Interaction between myelinated nerve fibres under nearly physiological conditions. 1. Observations on conduction velocity in single myelinated fibres. Jap. J. Physiol. **5:** 93–100, 1955

The impulse conduction velocity in a single fibre was studied in myelinated nerves of cold-blooded animals by stimulating the single fibre plus a few neighbouring ones. It was shown that the period of excitement in the isolated fibre was increased on stimulating adjacent fibres simultaneously. The conduction velocity in a given nerve section (12 cm) changed from 0·13 to 1·8 m/sec.

7. **BROWN, G. L.,** and **HOLMES, O.,** The effects of activity on mammalian nerve fibres of low conduction velocity. Proc. Roy. Soc. B**145**: No. 918, 1–14, 1956

The action of a series of stimuli on mammalian nerve fibres of low conduction velocity was studied. A monophasic action potential was noted in non-myelinated nerve fibres with speeds of less than 1·1 m/sec. Repeated stimulation of the low-velocity fibres caused an increased action current amplitude; in faster fibres the action currents did not change. An increase in action potential can be maintained for some time; in the opinon of the authors it is caused by hyperpolarization of the nerve by the repeated stimuli. The authors consider that the post-tetanic increase in action potential is a property common to all mammalian non-myelinated nerves; this explains post-tetanic facilitation in synaptic junctions.

8. **GOLOV, D. A.,** and **KOSTIUK, H. G.,** An input stage for an amplifier for detecting intracellular potentials. Fiziol. Zh. **42**: No. 1, 114–117, 1956

The circuit of a cathode follower required to connect the high-resistance micro-electrodes to the amplifier input is given. The intermediate cathode follower has a low input capacity and grid current.

Translated by J. J. OLIVER

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THE DIFFRACTION OF X-RAYS BY ORIENTATED MACROMOLECULAR MATERIALS*

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(*Received* 1 *December* 1956)

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THE X-ray pictures from many orientated polymers and fibrous proteins are similar to those from fine-ground, textured polycrystals. The likeness between the X-ray bands found with macromolecular materials and Debye powder patterns has on occasion caused polymers to be compared to aggregates of crystallites. This has influenced much X-ray work—attempts have been made to determine the unit cell and even total structural analyses (via atomic positions) have been attempted.

But the thermodynamic functions of such complex systems have been found to change smoothly during apparent phase transitions. So these systems show states typified by varying coexisting degrees of order. Physicochemical studies indicate that there should be no boundaries between structurally distinct regions in polymers. There are also no surfaces of separation between structurally uniform regions (as occur with polycrystals) which could determine the sorption properties of polymers. This means that to describe the structure of a polymer from crystallographic concepts alone omits some of its most important aspects, and to represent a polymer as an assembly of crystallites is simply incorrect. The work of Kargin and his school on this topic is well known [1].

It is desirable to emphasize those aspects of polymer structures which have to be studied in order to describe the structures completely. From the X-ray point of view a polymer must consist of coherent regions. So the structure of a polymer† must be such that the transition from one coherent area to another is continuous, no surface of separation being encountered. Smooth transitions between areas of coherent scattering are only possible if the structural packing of the polymer units is distorted relative to that which would exist in crystallites. The distortions can evidently be both dynamic and static, depending on the chain flexibility. Large distortions in polymer structures are compatible with the sharp drop in scattered X-ray intensity found on increasing the scattering angle.

Because of these distortions coherence must be retained in relation to elements close together, falling off gradually as the distance increases. The rate at which distortions accumulate determines the sizes of the coherent scattering regions and the degree of order in any given region. If the polymer was actually composed of true crystalline regions of sizes equal to those of the coherent scattering regions, a sharp step in the phase differences of X-rays scattered by adjacent areas would be found on passing between regions, as always occurs with a surface of separation.

† If the polymer is fibrous this naturally refers to the polymer fibril structure.

* *Biofizika*, **2**, No. 3, 281 [Reprint Order No. B10 34].

Here we suppose the phase differences to change continuously between regions via the gradual development of distortions. Any point can then be taken as the centre of some coherent scattering region.

The distortions may be distributed unevenly within the specimen, so we may speak of more and less ordered regions. The densities of the regions may vary. In some areas defects may accumulate relatively slowly. The sizes of the coherent scattering regions in these areas may then be so great that they give diffraction patterns equivalent to those of crystals. Then the X-ray methods used with polycrystals can sometimes give useful data on the mutual positions of the chain links in such structural areas. But such data cannot in any case be considered as exhausting the structural features of the polymer.

This model is compatible with physicochemical and X-ray data, but at present it is only predicted by the physicochemical data, the X-ray data in many cases indicating another model, namely one in which the passage between coherent regions occurs via displacement and rotation of whole regions, i.e. in essence a microcrystallite structure.

In our model of polymer structure, distortions in chain-link packing are most important. So if this type of model is to be assumed we have to consider the possible types and magnitudes of the distortions and their distributions in order to describe the structure completely. In deriving a model we should first consider the structures of certain polymers and fibrous proteins. To be able to study the distortions with X-rays we have to determine how certain structural defects must affect the form of, and intensity distribution in, the X-ray picture.

The nature of the defects can be partially ascertained by considering the diffraction of X-rays from various models of different types of distortion in the packing of the polymer elements. James [2] has indicated how to solve diffraction problems for chain structures. Kratky [3], Hosemann [4], Bear and Bolduan [5] and Whittaker [6] have given certain useful data on this.

The first such diffraction problem we considered was that of X-rays from a textured liquid. This is a system with chain molecules lying parallel, each chain showing long-range order. The chains themselves show liquid-type packing and have only short-range order.

This model may be described as follows in concrete geometrical terms:

(1) Definite atomic groupings repeat more or less regularly along each chain. The lengths of these groups remain constant along the chains for distances comprising many such units.

(2) The interchain distances are not constant but alter from pair to pair and from region to region. This is most conveniently dealt with via a function defining the probability of any interchain separation.

(3) The relative positions of the atomic groups in one chain bear no regular relation to those in another, i.e. the chains may be statistically displaced along their lengths and arranged in any way round the predominant axis.

This model is of course in a certain sense abstract. It relates to the extreme case where the order in the packing of parallel chain molecules is destroyed. In real polymers strong packing distortions are always due to deviations from chain parallelism in certain regions. Also when the chains are strictly parallel steric hindrance by

side-chains will to some extent restrict the angular and positional arrangements of equivalent groups in adjacent chains. But model studies of diffraction from textured liquids will enable one to estimate effects due to these structural distortions in any particular case. Clearly the results can also be used in studies on plastic deformation in orientated materials.

A general solution for the diffraction from such a model textured liquid will be given, and the results will be compared with experimental data on certain polymers

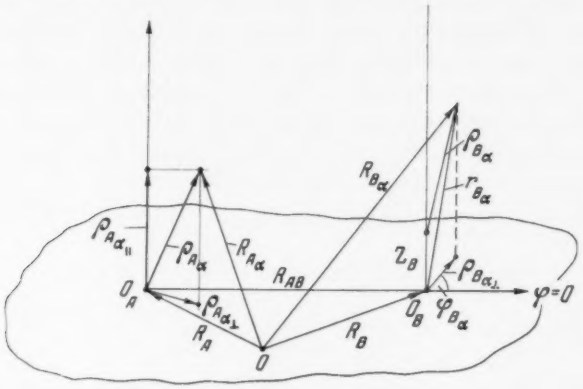

FIG. 1. Model for computing the diffraction pattern. In the model shown $Z_A = 0$ so $\rho A\alpha = rA\alpha$.

and proteins. The possibility of deriving the packing distribution function for chain molecules from the intensity curve via a Fourier–Bessel integral transform will also be demonstrated. The method is, however, correct for any degree of order in the system if it has this packing. This problem is analogous to that of Debye, Zernicke and Prins for the scattering from liquids and amorphous substances in the particular case of parallel chain molecules and for systems showing cylindrical symmetry on the macro scale.

Let us consider a system consisting of N parallel chains. For convenience we divide the system by a plane perpendicular to the molecular axes passing through the origin O for the whole system, and through the origins for the separate molecules (O_A, O_B, etc. see Fig. 1). The amplitude of the wave scattered by molecule A in the direction specified by the vector $\bar{S}'$ will proportional to

$$\sum_{\alpha=1}^{n} f_\alpha \exp\left(-i\chi \bar{S}\bar{R}_{A\alpha}\right) \tag{1}$$

where f_α is the scattering power of the αth atom, $R_{A\alpha}$ is the distance of atom α from the origin, $\bar{S}$ being a vector directed along the resultant of the unit vectors for the incident and reflected rays, $\bar{S}'-\bar{S}'_0 = \bar{S}$, $|\bar{S}| = 2\sin\theta$ where θ is half the scattering angle, $\chi = {}^{2\pi}/_{\lambda}$ being the wavenumber, and n the number of atoms in the molecule.

For the whole system of molecules

$$E \sim \sum_{A=1}^{N}\sum_{\alpha=1}^{n} f_\alpha \exp\left(-i\chi \bar{S}\bar{R}_{A\alpha}\right). \tag{2}$$

The scattered intensity is proportional to

$$I \sim \sum_{A=1}^{N}\sum_{B=1}^{N}\sum_{\alpha=1}^{n}\sum_{\beta=1}^{n} f_\alpha f_\beta \exp(-i\chi\bar{S}\bar{R}_{A\alpha}) \exp(i\chi\bar{S}\bar{R}_{B\beta}). \quad (3)$$

Replacing $\bar{R}_{A\alpha}$ by $r_{A\alpha}-\bar{R}_A$, $\bar{R}_{B\beta}$ by $r_{B\beta}-\bar{R}_B$ and putting $\bar{R}_{AB}=\bar{R}_B-\bar{R}_A$ we get

$$I \sim \sum_{A}\sum_{B}\sum_{\alpha}\sum_{\beta} f_\alpha f_\beta \exp(-i\chi\bar{S}\bar{R}_{AB}) \exp(-i\chi\bar{S}\bar{r}_{A\alpha}) \times \exp(i\chi\bar{S}\bar{R}_{B\beta}). \quad (4)$$

This equation gives the scattered X-ray intensity from any assembly of molecules. Let us divide into two parts, corresponding to $A=B$ (intramolecular scattering) and $A \neq B$ (intermolecular diffraction). This division is permissible in all cases. Deviations from the true crystal structure are here dealt with by considering each term in the sum separately and altering each one independently, as required by the structural distortions. The first term in the equation can be transformed because the molecules contain groups which repeat periodically along their lengths. Then $\bar{r}_{A\alpha} = m\bar{c} + \bar{\rho}_{A\alpha}$, where m is an integer, $\bar{c}$ the chain period and $\rho_{A\alpha}$ the radius vector of the αth atom within a period.

If p is number of atoms in a periodically repeating group then

$$\sum_{\alpha=1}^{n} f_\alpha e^{-i\chi\bar{S}\bar{r}_{A\alpha}} \cdot \sum_{\beta=1}^{n} f_\beta e^{i\chi\bar{S}\bar{r}_{A\beta}} = \left[\sum_{m} e^{-i\chi\bar{S}m\bar{c}}\right]^2 \cdot \sum_{\alpha=1}^{p} f_\alpha e^{-i\chi\bar{S}\bar{\rho}_{A\alpha}} \times \sum_{\beta=1}^{p} f_\beta e^{i\chi\bar{S}\bar{\rho}_{A\beta}} = L'F^2. \quad (5)$$

Here L' is the one-dimensional Laue function, differing from zero only when the projection of $\bar{S}$ on the fibre axis is $\lambda l/c$ where l is an integer and F is $\sum f_\alpha \exp(-i\chi\bar{S}\bar{\rho}_{A\alpha})$ taken over all the atoms in the repeating group, being the structure factor for the molecule. For any other molecule with the same orientation relative to the texture axis but displaced in the z-direction (fibre axis) relative to A, the expression for the scattered intensity will be the same, since displacements in z only alter the scattering phase of a molecule, while we are here summing over intensities and not amplitudes. For the same reason we should average F^2 when averaging the first term over all possible orientations about the texture axis. Then the first term in the sum will be

$$NL'F^2. \quad (6)$$

The second term can also be transformed. Consider the expression

$$\sum_{A}\sum_{B}\sum_{\alpha}\sum_{\beta} f_\alpha f_\beta \exp(-i\chi\bar{S}\bar{R}_{AB}) \exp(-i\chi\bar{S}\bar{r}_{A\alpha}) \times \exp(i\chi\bar{S}\bar{r}_{B\beta}) \quad (7)$$

when $A \neq B$. The relative displacements and independent orientations of the molecules about the texture axis must be considered. We note that $\bar{r}_{A\alpha} = m\bar{c} + \bar{\rho}_{A\alpha}$, and $\bar{r}_{B\alpha} = m\bar{c} + \bar{\rho}_{B\alpha} + (\bar{Z}_B - \bar{Z}_A)$ where $\bar{Z}_B - \bar{Z}_A$ is the displacement of B relative to A.

From this (7) equals

$$L' \sum_{B}\sum \overline{F_A F_B^*}\, e^{-i\chi\bar{S}\bar{R}_{AB}}\, \overline{e^{-i\chi\bar{S}(\bar{Z}_B-\bar{Z}_A)}},$$

$$\text{where } F_A = \sum_{\alpha=1}^{p} f_\alpha e^{-i\chi\bar{S}\bar{\rho}_{A\alpha}},\ F_B = \sum_{\beta=1}^{p} f_\beta e^{-i\chi\bar{S}\bar{\rho}_{B\beta}}$$

and F_B^* is the complex conjugate of F_B. Since the projections $\bar{\rho}_{A\alpha}$ and $\bar{\rho}_{B\alpha}$ on the fibre axis are equal, and the perpendicular projections are of equal modulus but have different orientations, then

$$F_A = \sum_{\alpha=1}^{p} f_\alpha e^{-i\chi[S_\perp \rho_{\alpha_\perp} \cos\varphi_{A\alpha} + S_\parallel \rho_{\alpha_\parallel}]},$$

$$F_B = \sum_{\beta=1}^{p} f_\beta e^{-i\chi\,[S_\perp \rho_{\beta\perp} \cos\varphi_{B\beta} + S_\parallel \rho_{\beta\parallel}]}.$$

Here $S_\parallel$ and $S_\perp$ are the projections of $\bar{S}$ on the axis and the plane normal to it, $\varphi_{A\alpha}$ and $\varphi_{B\beta}$ being the difference of azimuth angles for $\bar{S}$ and $\bar{\rho}_{A\alpha}$ and for $\bar{S}$ and $\bar{\rho}_{B\beta}$ relative to the chosen co-ordinate system, $\rho_{\alpha\perp}$ $\rho_{\beta\perp}$ $\rho_{\alpha_\parallel}$ and $\rho_{\beta_\parallel}$ being the moduli of the projections of $\bar{\rho}_{A\alpha}$ and $\bar{\rho}_{B\beta}$.

The general expression for the scattered intensity is then

$$I \sim L' \,[N\bar{F}^2 + \sum_A \sum_B F_A F_B^* \, e^{-i\chi\bar{S}\bar{R}_{AB}} \, e^{-i\chi\bar{S}(\bar{Z}_B \bar{Z}_A)}] \tag{8}$$

The common factor in (8) is the one-dimensional Laue function. This determines the positions of the maxima on the layer lines, which satisfy $S/\lambda = l/c$, where l (an integer) is the number of the layer line.

The mean intensity due to each molecule independently can be computed by averaging F^2 over all possible orientations of $\bar{\rho}$ relative to $\bar{S}$. Resolving $\bar{S}$ and $\bar{\rho}$ into two components (parallel and normal to the fibre axis) we have

$$F^2 = \sum_\alpha \sum_\beta f_\alpha f_\beta e^{-i\chi\bar{S}\bar{\rho}_{\alpha\beta}} = \sum_\alpha \sum_\beta f_\alpha f_\beta e^{-i\chi[S_\perp \rho_{\alpha\beta_\perp} \cos\varphi^A_{\alpha\beta} + S_\parallel \rho_{\alpha\beta\parallel}]},$$

where $\varphi^A_{\alpha\beta}$ is the difference in azimuthal angles relative to some chosen direction normal to the fibre axis for $\bar{\rho}_{\alpha\beta\perp}$ and $\bar{S}$, A being the molecular index. Then

$$\bar{F}^2 = \frac{1}{2\pi}\int_0^{2\pi} \sum_\alpha \sum_\beta f_\alpha f_\beta e^{-i\chi[S_\perp \rho_{\alpha\beta_\perp} \cos\varphi + S_\parallel \rho_{\alpha\beta\parallel}]} \, d\varphi$$

$$= \sum_\alpha \sum_\beta f_\alpha f_\beta \mathcal{J}_0(\chi S_\perp \rho_{\alpha\beta\perp}) e^{-i\chi S_\parallel \rho_{\alpha\beta_\parallel}}$$

and the term defining the intramolecular scattering is

$$NL' \sum_\alpha \sum_\beta f_\alpha f_\beta \mathcal{J}_0(\chi S_\perp \rho_{\alpha\beta\perp}) \, e^{-i\chi S_\parallel \rho_{\alpha\beta_\parallel}}. \tag{9}$$

Here $\mathcal{J}_0(\chi S_\perp \rho_{\alpha\beta_\perp})$ is the zero-order Bessel function.

The mean fractional intensity due to each chain pair can be found by averaging $F_A F_B^*$ over all possible orientations of $\bar{\rho}_\alpha$ and $\bar{\rho}_\beta$ relative to $\bar{S}$, and $\exp[-i\chi\bar{S}(\bar{Z}_B - \bar{Z}_A)]$ over all possible $(\bar{Z}_B - \bar{Z}_A)$. Further, $\exp(-i\chi\bar{S}\bar{R}_{AB})$ must be multiplied by the probability of the chains being a distance $\bar{R}_{AB}$ apart, and integrated over all possible distances.

On averaging $F_A F_B{}^*$ over all possible orientations we must take the orientations of all molecules as being independent; then

$$\overline{F_A F_B^*} = \overline{\sum_{\alpha=1}^{p} f_\alpha e^{-i\chi\bar{S}\bar{\rho}_{A\alpha}}} \; \overline{\sum_{\beta=1}^{p} f_\beta e^{-i\chi\bar{S}\bar{\rho}_{B\beta}}} = \left[\sum_\alpha f_\alpha I_0(\chi S_\perp \rho_\alpha) \, e^{-i\chi S_\parallel \rho_{\alpha_\parallel}}\right]^2 = (\bar{F})^2. \tag{10}$$

The mean of $\exp[-i\chi\bar{S}(\bar{Z}_B - \bar{Z}_A)]$ is determined via $\frac{1}{c}\int_0^c \exp(-i\chi\bar{S}\bar{Z}')d\bar{Z}'$, since $\bar{Z}_B - \bar{Z}_A = \bar{Z}'$ can only take any value in the range 0 to c. Since the maxima from intermolecular diffraction must always coincide in position with the maxima in the

one-dimensional Laue function it is meaningful to consider this integral only when $S = \lambda l/c$, l being an integer (including zero). Since the product of the normal component of $\bar{S}$ by $\bar{Z}$ is zero the integral takes the form

$$\frac{1}{c}\int_0^c e^{-i\chi\bar{S}\bar{Z}'}\,dZ' = \frac{1}{c}\int_0^c e^{-\frac{2\pi i l Z'}{c}}\,dZ'.$$

But $\int_0^c \exp\left(-\frac{2\pi i l z'}{c}\right) dZ' = 0$ when $l \neq 0$ and 1 when $l = 0$.

So except for the equatorial layer line, the second term in (8) is zero, and the intensity is determined by the independent scattering by all the molecules in the system. James [2] derived this result from qualitative arguments.

Let us consider the intensity distribution along the equatorial layer line. Then $S_{\|} = 0$ and $\overline{\exp(-i\chi\bar{S}\bar{Z}')} = 1$. Let us find the mean of $\exp(i\chi\bar{S}\bar{R}_{AB})$. Let $[W(R_{AB})\,dQ_A dQ_B Q^2]$ define the probability that A and B simultaneously intersect the areas dQ_A and dQ_B, i.e. the probability that the chains are $\bar{R}_{AB}$ apart (here W is the probability density function).

The second sum in (8) contains $N(N-1)$ terms. Hence it becomes

$$\frac{N(N-1)}{Q^2} L'(\bar{F})^2 \iint W(R_{AB}) e^{-i\chi\bar{S}\bar{R}_{AB}}\,dQ_A dQ_B.$$

W determines the mean values over the whole of space, so it must be radially symmetric relative to any chain as origin. With an arbitrary arrangement of molecules with any $R > R_0$, where R_0 is the radius of a molecule, $W = 1$. When R is large W is also unity. The second term can be transformed in the normal way when dealing with diffraction from liquids, amorphous substances and crystalline powders. In view of its properties W can conveniently be replaced by $1-(1-W)$ in the integrand. So we get the difference of two integrals

$$\frac{c^2}{Q^2}\iint e^{-i\chi\bar{S}\bar{R}_{AB}}\,dQ_A dQ_B - \frac{c^2}{Q^2}\iint (1-W)\, e^{-i\chi\bar{S}\bar{R}_{AB}}\,dQ_A dQ_B,$$

where

$$c^2 = L'(\bar{F})^2 N(N-1). \tag{11}$$

Consider the first of these. It is analogous to the term which determines the zero scattering. Since it corresponds to an arbitrary molecular arrangement it can be written as the product of two integrals. Using cylindrical co-ordinates we get the following forms for the integrals

$$\frac{c}{Q}\int_0^{R_1}\int_0^{2\pi} e^{-i\chi\bar{S}\bar{R}}\,RdRd\varphi \text{ and } \frac{c}{Q}\int_{R_1}^{R_1}\int_0^{2\pi} e^{-i\chi\bar{S}\bar{R}}\,RdRd\varphi. \tag{12}$$

Here R_1 is the radius of the specimen, R_0 the mean radius of a chain.

In the first case the integration is carried over the whole cross-section of the specimen, whereas in the second the cross-sectional area of the first chain is considered:

$$\frac{c}{Q}\int_0^{R_1}\int_0^{2\pi} e^{-i\chi\bar{S}\bar{R}}\,Rd\varphi dR = \frac{2\pi c}{Q}\int_0^R \mathcal{J}_1(\chi S_{\perp} R)\,RdR$$

$$= \frac{2\pi c R_1}{Q\chi S_\perp} \mathcal{J}_1(\chi S_\perp R_1 = \frac{2c}{\chi S_\perp R_1} \mathcal{J}_1(\chi S_\perp R_1) \text{ or } \frac{2c\mathcal{J}_1(x)}{(x)},$$

where $x = \chi S R_1$.

The second integral can be put as the difference of two, taken between the limits 0 to R_1 and 0 to R_0. The latter is equal to $\frac{2R_0 c}{\chi S_\perp R_1^2} \mathcal{J}_1(\chi S_\perp R_0)$, being practically zero on account of the factor R_0/R_1^2. So both integrals become

$$\frac{2c\mathcal{J}_1(x)}{x} \tag{13}$$

Fig. 2 shows $\frac{2\mathcal{J}_1(x)}{x}$. It is very similar to $\varphi(x) = \frac{3(\sin x - x\cos x)}{x^3}$, which occurs in diffraction theory. Its first zero occurs when the argument is 3·832, the maxima then dying away rapidly. Since here R_1 is the radius of the whole scattering volume, it is clear that the intensity defined by this term will differ from zero only when $S_\perp$ is very close to zero. When $S_\perp = 0$ each of the integrals is unity, and this term in the sum is

$$N(N-1)\, L'\, (\bar{F})^2. \tag{14}$$

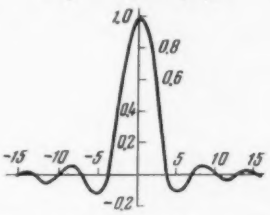

FIG. 2. Function $\frac{2\mathcal{J}_1(x)}{x}$ showing the distribution intensity at the zero maximum of a textured liquid.

Since the probability of finding chain A in any element of volume is everywhere the same, the integration over dQ_A in

$$-\frac{c^2}{Q}\int\int(1-W)\, e^{-i\chi\bar{S}\bar{R}_{AB}}\, dQ_A dQ_B$$

gives Q, and the integral is determined as follows:

$$\frac{N(N-1)}{Q} L'(\bar{F})^2 \int (1-W)\, e^{-i\chi\bar{S}\bar{R}}\, dQ$$

$$= \frac{2\pi N(N-1)}{Q} L'(\bar{F})^2 \int_0^{R_1} (1-W)\, \mathcal{J}_0(\chi S_\perp R) R dR. \tag{15}$$

Since as R increases W approaches 1 rapidly, the integration can be taken from 0 to ∞. The final expression is then

$$I \sim NL'\left[\bar{F}^2 - \frac{2\pi(N-1)}{Q}(\bar{F})^2 \int_0^\infty (1-W)\, \mathcal{J}_0(\chi S_\perp R) R dR\right]. \tag{16}$$

Frequently W can be more conveniently put in another form. The number of molecules passing through unit cross-sectional area at a distance R from the chain is

$\frac{NW(R)}{Q} = \delta(R)$. Replacing W by the density distribution function and $N-1$ by N, which is possible when N is large, we get

$$I \sim NL'\left\{\bar{F}^2 + 2\pi(\bar{F})^2 \int_0^\infty [\delta(R)-\delta_0]\mathcal{J}_0(\chi S_\perp R)RdR\right\}. \tag{17}$$

This relation is analogous to those for X-ray diffraction in amorphous materials, liquids, gases and crystal powders for the cases of parallel chain molecules and systems showing cylindrical symmetry on the macro scale. But it only gives the equatorial intensity distribution because the model considered is one-dimensional. When $S_\parallel$ is non-zero the intensity is given by

$$I \approx NL'\bar{F}^2 = NL' \sum_\alpha \sum_\beta f_\alpha f_\beta \mathcal{J}_0(\chi S_\perp \rho_{\alpha\beta\perp})\, e^{-i\chi S_\parallel \rho_{\alpha\beta\parallel}}.$$

This solution can be shown to imply that the intensities of all layer lines, barring the zeroth, are determined solely by the intramolecular scattering, i.e. by $NL'\bar{F}^2$— in essence by $\bar{F}^2$. The term for the intermolecular scattering is everywhere zero except on the equator because the chains show no order along their lengths. F^2 has subsidiary maxima, so the layer-line reflections will form extended bands. The intensities on the meridian are given by

$$I \sim NL'p^2 \sum_\alpha \sum_\beta f_\alpha f_\beta e^{-i\chi S_\parallel \rho_{\alpha\beta\parallel}},$$

where p is the number of atoms in the periodically repeated group. The meridional maxima may be suppressed by the factor

$$\sum_\alpha \sum_\beta f_\alpha f_\beta \exp\left(-i\chi S R_{\parallel} \rho_{\alpha\beta\parallel}\right)$$

on certain layer-lines. The band intensities must fall off away from the meridian, to correspond with distribution of maxima in $\mathcal{J}_0$ (Fig. 3).

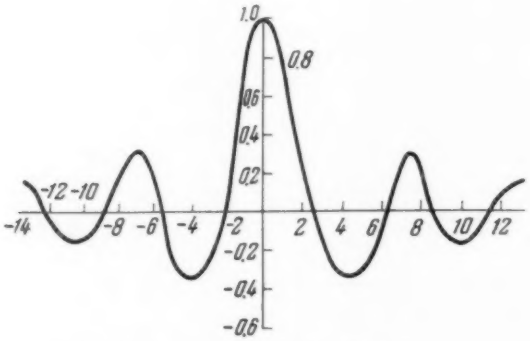

FIG. 3. Graph of the function $\mathcal{J}_0(x)$.

The intensity distribution on the equator will be determined by W as well as by $\bar{F}^2$. So the diffuseness of the equatorial reflections may be different (it can be less). The diffuseness found by direct observation of course depends on the angle of

reflection. At relatively small angles the reflections may appear fairly sharp, while their angular spread may be large. The rate of intensity fall-off in the equatorial reflections is also determined by W, which includes the deviations in the interchain distances. If the chain-packing is of "liquid" type, two to three diffuse reflections will be found on the equator, the intensity near the centre being weakened. With gas-type packing the intensity falls gradually from the centre outwards, and so we find streaks passing through the centre.

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Fig. 4

Fig. 5

Fig. 6

FIG. 4. Model diffraction patterns for a textured liquid when individual regions are only slightly disorientated.

FIG. 5. Diffraction pattern of nylon [9].

FIG. 6. Diffraction pattern of treated cellulose hydrate [10].

A slight disorientation in the various sections of a specimen, which is universal, must particularly affect the shapes of the equatorial reflections. The short equatorial reflection lines are thereby spread out into diffuse spots. But the meridional reflections are almost unaffected.

As long-range order exists along the chains the diffraction pattern must extend further along the meridian than along the equator.

Fig. 4 is a model of the diffraction pattern from a textured liquid. Figs. 5, 6 and 7 show patterns for nylon [7], mercerized cellulose [8] and an α-group fibrous protein. The patterns from these polymers have many resemblances to that of our model. The sharp one-dimensional diffraction bands indicate that the molecules are not regularly arranged along their lengths. The sharp drop in intensity on the equator indicates large distortions in molecular packing. The chain orientations are highly ordered.

The diffraction patterns of some of these substances show reflections of type additional to the textured liquid ones. These can be considered as due to scattering in areas not corresponding to the above model.

Unfortunately the diffraction patterns reproduced in the original articles do not give a sufficient idea of the anistropy in the diffraction from these materials. But we know the α-group proteins to give extremely anisotropic patterns. There are at most

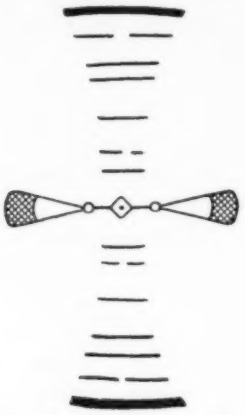

FIG. 7. General pattern found with α-group fibrous proteins.

one to two equatorial diffuse maxima with these, having $(\sin\theta/\lambda_{max}) \sim 0{\cdot}05$, while fairly strong meridional reflections with $(\sin\theta/\lambda) \sim 0{\cdot}3$ are found [2]. Some α-group proteins also giving streaks passing through the central spot. The directions of the intrachain hydrogen bonds retain their periodic arrangement while the molecular packing is extremely strongly distorted due to structural differences in the side-groups: this must cause the intensity to fall as $S_\perp$ increases, i.e. as we leave the meridian.

In conclusion it is of value to consider the general principles for determining probability functions for the spacing of chain molecules. These principles are applicable to highly ordered systems of structural elements in addition to textured liquids. When the statistical shift and repetition of the chains is non-arbitrary the equatorial intensity is

$$I \sim NL'F^2\left[1+2\pi\int_0^\infty [\delta(R)-\delta_0]\,\mathcal{J}_0(\chi S_\perp R)\,RdR\right].$$

$\delta_0-\delta(R)$ specifies any type of packing from true crystalline to pure gaseous. To pass from I to $\delta_0-\delta(R)$ we need only know the system to be cylindrically symmetrical. The requisite formula is deducible from the Fourier–Bessel integral theorem.

Only the equatorial intensity distribution is considered.

Denote $\dfrac{2\pi(S-1)}{Q}\cdot\int_0^\infty (1-W)\,\mathcal{J}_0(\mu R)\,RdR$ by $i(\mu)$. Here $\mu = \chi S_\perp = \dfrac{4\pi\sin\theta}{\lambda}$

Evidently $i(\mu) = \dfrac{I_m - I_M}{L'(F)^2} = \dfrac{I_m}{L'(F)^2}\left(1-\dfrac{I_M}{I_m}\right) = \dfrac{\bar{F}^2}{(F)^2}\left(1-\dfrac{I_M}{I_m}\right)$, (18)

where $I_M = I/N$, $I_m = L'F^2$.

It is here more convenient to represent $W(R)$ as a density distribution function, so

$$i(\mu) = 2\pi\int_0^\infty [\sigma(R)-\sigma_0]\,\mathcal{J}_0(\mu R)\,RdR, \qquad (19)$$

and $$\varphi(R) = \sigma(R) - \sigma_0$$

is a function continuous in the range $R_0 < R < R_1$ (here R_0 is the mean chain radius) having a finite number of maxima and minima in this range. So $i(\mu)$ is of the form

$$c \int_0^{\infty} \varphi(R)\, \mathcal{J}_n(\mu R)\, R dR,$$

where $\varphi(R)$ satisfies Dirichlet's conditions.

The Fourier–Bessel integral theorem gives

$$\int_0^{\infty} d\mu \int_0^{p} \mu R \varphi(R)\, \mathcal{J}_n(\mu R)\, \mathcal{J}_n(\mu R\alpha) = \varphi(R\alpha).$$

so

$$\int_0^{\infty} \mu i(\mu)\, \mathcal{J}_0(\mu R\alpha)\, d\mu = 2\pi [\sigma(R\alpha) - \sigma_0]. \tag{20}$$

It is more convenient to determine the mean number of chains at a given distance from the reference chain, i.e. to determine

$$2\pi R\alpha\sigma(R\alpha) = 2\pi R\alpha\sigma_0 + R\alpha \int_0^{\infty} \mu i(\mu)\, \mathcal{J}_0(\mu R)\, d\mu. \tag{21}$$

This is the final formula, which can be used for calculations. The method can be used to determine packing peculiarities in well-orientated polymers. A necessary requirement before the method can be used is that we must know the molecular structure factor, which latter is now known fairly accurately for a number of polymers. It can be computed for certain compounds of high molecular weight if we used the theory Mizushima *et al.* [10] have developed. The method can sometimes give useful data on the packing of chain molecules in macromolecular substances.

In conclusion the authors take the opportunity of thanking Iu. A. Bagariatskii, doctor of physicomathematical sciences, for his valuable advice and comments.

CONCLUSIONS

(1) A general structural model for orientated macromolecular substances subject to various types of packing distortion is considered. In this model the dimensions of the coherent regions are conditioned by the rate of accumulation of distortions and not by dividing surfaces; the passage from one area to another is continuous. This model agrees better with physicochemical data than does one in which the orientated polymer is considered as an aggregate of crystallites.

(2) The model is used to analyse a first concrete case corresponding to disordering of parallel chain molecules (textured liquid model). This model approximates to the structures of areas in certain polymers and fibrous proteins.

(3) A solution for X-ray scattering by a textured liquid is derived which is analogous to Debye and Zernicke's problem for a system of cylindrical symmetry. It is shown that $W(R)$ can be determined by using a Fourier–Bessel integral transform on the

intensity. The latter transform is applicable to a parallel chain molecule system with any degree of order.

(4) The theoretical results are compared with experimental data for certain polymers and fibrous proteins (nylon, mercerized cellulose and α-group fibrous proteins). The structural packing in these materials is shown to correspond with a one-dimensional model.

Translated by J. E. S. BRADLEY

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ON THE STRUCTURE OF MOLECULAR CHAINS OF COLLAGEN*

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(*Received* 1 *December* 1956)

STUDIES in the geometry of polypeptide chains are basic to structural investigations on proteins. Considerable progress has already been made and a number of possible equilibrium configurations for polypeptide chains have been discovered. These include the well-known α-helix model for certain proteins. Models have also been constructed and tested for the β-subgroup proteins. But these models do not exhaust the possible structures. X-ray data indicate the existence of other configuration types not comprised in the models.

The structure of collagen is of considerable interest in this connexion. Collagen gives a comparatively good X-ray pattern, indicating that a special chain-ordering arrangement exists in certain areas [1, 2]. Also the wide variety of collagens with various physicochemical properties, which frequently relate to various structural differences, would seem to indicate that some of the X-ray data might be given a rational explanation.

The structure of collagen has long attracted attention. The first models were constructed over 20 years ago, and 15 such models have so far been proposed. Latterly a model in very good agreement with the X-ray data and many of the physicochemical properties has been proposed. But none of these models is as

* *Biofizika*, **2**, No. 3, 294 [Reprint Order No. BIO 35].

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TABLE 1. AMINO ACID COMPOSITIONS OF COLLAGEN GROUP PROTEINS (IN g RESIDUE PER 100 g PROTEIN)

Amino acid	Various mammal collagens				Gelatin			Icthyocol	Fish-scale protein	Ictholepidin	Cornein		Rat-tail tendon collagen	Rabbit skin procollagen		Pike-skin collagen	Cod-skin collagen	Spongin	
(Reference)	[3]	[4]	[5]	[6]	[7]	[3]	[8]	[7]	[8]	[8]	[9]	[10]	[11]	[12]	[11]	[13]	[13]	[10]	[14]
Glycine	—	19·9	26·2	20·2	27·0	—	23·6	9·5	20·6	—	—	15·5	22·9	21·2	19·8	—	—	14·4	—
Alanine	—	7·6	9·5	8·2	8·7	—	—	14·7	—	—	—	—	9·8	6·5	7·9	—	—	0·2	—
Phenylolanine	2·5	3·7	4·2	1·98	2·6	2·2	2·1	1·8	2·9	—	—	5·7	4·2	1·8	1·8	—	—	3·3	—
Leucine	3·5	4·8	3·2	3·2	3·3	3·2	3·1	3·8	3·6	—	—	—	3·2	—	2·8	—	—	6·1	—
Valine	16·9	2·9	3·4	2·1	2·5	17·6	2·8	2·3	2·9	—	—	—	3·7	—	1·9	—	—	—	—
Proline	10·5	12·7	15·1	12·2	17·5	18·0	15·3	17·8	8·6	—	—	—	14·6	—	11·0	13·3	10·8	4·3	5·7
Hydroxyproline	—	12·1	14·0	11·1	14·6	—	—	4·7	—	—	—	—	13·0	16·4	11·7	7·9	5·8	0·0	0·0
Glutamine	—	10·0	11·3	9·7	5·8	11·5	8·4	8·2	7·2	—	—	—	11·2	8·7	9·7	—	—	14·6	—
Asparagine	6·9	5·5	6·3	6·0	3·4	6·7	—	4·7	—	—	—	—	6·0	4·0	5·2	—	—	3·5	—
Arginine	8·0	7·9	8·8	7·4	8·9	8·0	8·0	9·3	6·7	5·4	6·4	4·5	6·6	6·7	7·4	—	—	4·3	—
Lysine	3·5	4·0	4·5	3·5	5·9	4·1	5·0	4·3	3·7	3·7	2·7	3·3	4·5	3·5	3·1	—	—	3·0	—
Hydroxyproline	—	1·1	1·3	—	0·9	—	—	0·3	—	—	—	—	1·3	—	—	—	—	—	—
Histidine	—	0·7	0·8	0·6	0·9	0·8	1·0	1·1	2·5	3·1	0·5	0·9	0·3	1·9	0·3	—	—	0·2	—
Serine	—	2·7	3·4	3·5	2·7	—	3·4	4·1	7·5	—	—	—	3·3	—	3·5	—	—	—	—
Threonine	2·5	2·0	2·4	—	1·4	1·9	0·8	3·2	2·8	—	—	—	2·2	—	—	—	—	—	—
Methionine	0·9	0·7	0·8	0·8	1·0	1·0	0·8	2·8	2·8	3·0	—	—	0·7	0·5	0·7	—	—	—	—
Cystine	0·3	0·0	0·0	0·0	0·1	0·07	0·1	0·1	0·7	1·1	5·5	9·0	0·0	0·0	—	—	—	2·8	—
Tyrosine	0·9	1·3	1·4	0·9	0·0	0·4	0·7	0·0	2·8	5·2	—	13·0	0·9	0·0	0·1	—	—	0·8	—
Tryptophane	—	0·0	0·0	0·0	0·0	—	0·0	0·0	0·3	0·6	—	0·0	0·0	0·0	—	—	—	0·0	—
*Iso*leucine	1·9	—	2·1	1·6	1·7	1·4	1·7	3·0	1·9	—	—	—	2·1	—	—	—	—	—	—

convincing as the α-helix model for the α-group proteins. In our view the main fault is that there is no real basis for the assumptions made about the groups responsible for the specific chain configurations. The models are purely hypothetical, and no reason for the specific polypeptide chain configuration in collagen has yet been advanced. We consider the elucidation of this the primary object of structural studies on collagen.

Various hypotheses are possible. We can first assume that the chain configuration is not related to the definite amino acid contents, but is due only to the properties of the separate chain segments, as in the α-group proteins. We suppose the configuration to occur in regions not containing imino acids, or where the amino acid and imino acid residues occupy equivalent positions. Since at present we have little information of polypeptide chain stereochemistry, we cannot assume that this does not happen. Then the overall amino acid compositions of proteins giving collagen-type X-ray patterns could vary over a large range, and the fraction of the chain length having this specific configuration should not depend on the content of any amino acid residue.

On the other hand, we may suppose that the configuration is determined by some definite amino acid residues. If the residues are assumed randomly distributed then if some are to determine the chain configuration they must predominate in amount. Thus almost all the chain should adopt the special configuration, and only at particular points should it be disturbed by interaction between the groups in different chains. If we assume a non-random distribution, the special configuration should occur in those parts of the chain where the amino acids which determine the configuration accumulate [2]. In this case the fraction of the length showing the special configuration should depend on the amino acid composition.

Let us consider the chemical data on the collagen-type proteins. Consider first the overall amino acid compositions. Table 1 shows that there is no regularity in the amino acid contents which could give rise to the special chain configuration. The group tends to show high imino acid, glycine and polar amino acid contents. But some collagen-type proteins do not. Spongin, for instance, contains very little imino acid. Some non-collagen proteins have high polar amino acid contents; there are also such proteins having much glycine and imino acids. The data of Table 2 on the imino acid and glycine contents of some proteins are of interest. Elastin, for

Table 2. Occurrence of certain amino acids in some proteins

Protein	Proline (per cent)	Hydroxy-proline (per cent)	Glycine (per cent)	X-ray pattern type
Spongin	5	0	14·4	collagen
γ-globulin	6·8	—	3·2	—
Elastin	13·2	2	29·4	—
β-casein	13·5	—	1·8	—
Pike-skin collagen	13·3	7·9	—	collagen
Cod-fish skin collagen	10·8	5·8	—	collagen
Procollagen	10·98	11·75	19·8	collagen
RTT collagen	14·66	13	22·9	collagen

instance, which does not show the collagen structure, has high glycine and imino acid contents. The very high proline and hydroxyproline contents of spongin resemble those of γ-globulin, but spongin has the collagen chain.

The data show that no statistical arrangement of amino acid residues can account for the collagen structure. The groups responsible for the structure might be from imino acids, glycine and the polar amino acids. But the imino acid content varies too widely (from 5 to 30 per cent). The same could be said of glycine. There are also proteins with many such polar groups which do not show this special configuration.

Thus two hypotheses are possible:

(1) The configuration occurs in distinct areas, and is due to the accumulation of specific groups (the total chain length having the special configuration depending on the specific amino acid contents);

(2) The configuration occurs in distinct areas, and is one of the possible equilibrium forms for the polypeptide chain (the total chain length having the specific configuration not depending on the content of any amino acid).

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The numbers and sizes of the chain sections can be determined from the integral intensities and heights of the interference maxima. By studying how these depend on the amino acid composition for various collagens we may be able to decide in favour of one of the hypotheses. The present paper deals with the relation of X-ray diffraction pattern to amino acid composition for collagens.

EXPERIMENTAL

We used spongin, pike- and cod-skin collagen, rabbit-skin procollagen and RTT collagen. Their imino acid and glycine contents vary within wide limits (Table 1).

The sponging was derived from sea-sponges, which consist of spongin plus mineral inclusions. The mineral inclusions were removed with 5 per cent HCl. Layers consisting of collagen fibres were separated from the cod- and pike-skin. The specimens were washed in distilled water and were electrodialysed for two days to remove inorganic contaminants. Orekhovich's method [12] was used for procollagen. RTT collagen was produced in the usual way. For our purpose it was sufficient to remove crystalline inclusions. Other contaminants merely increased the background; in all cases the films were normalized from the overall elementary composition. The specimens were carefully disorientated by fine shredding and prepared as rods of diameter not more than 1 mm.

The elementary compositions were determined,* except for procollagen, the published data being used for the latter [12] (see Table 3).

In all cases we used CuKα radiation, monochromatized by reflection from a flat single crystal of pentaerythritol in a special monochromator [15]. Agfa and XX films were used in planar and cylindrical cameras, the exposure times being from 1 to 2 hr at 37 kV and 15 mA. Up to five films were taken from each specimen in the cylindrical chamber. To study spots near the primary beam films were taken with a flat cassette.

The intensities were measured on an MF-4 microphotometer. Measurements

* Determined in the microanalysis laboratory at the Faculty of Chemistry, Moscow State University.

were made at fixed slit widths at 0·25 mm intervals. Ten complete intensity curves were taken for each specimen using several plates for areas near the primary beam.

TABLE 3. ELEMENTARY PERCENTAGE COMPOSITION OF SOME COLLAGENS

Specimen	C	O	N	H
Collagen	42·6	34·9	15·2	7·3
Procollagen	51·0	24·8	16·9	7·3
Pike-skin collagen	42·5	36·2	13·7	7·6
Cod-skin collagen	42·6	35·3	14·6	7·5
Spongin	40·6	39·8	12·7	6·9

The curves were reduced to absolute units. Each intensity, corrected for polarization in the large-angle region ($\sin\theta/\lambda \sim 0{\cdot}64$) was equated to the sum of coherent and incoherent scattering contributions computed from the elementary composition. No absorption corrections were made, since calculation shows them small. The mean absolute curve was drawn for each specimen. By reducing the data to absolute units allowance for the difference in scattering volumes could be made. The data were also used to normalize the flat-film measurements using a limited field.

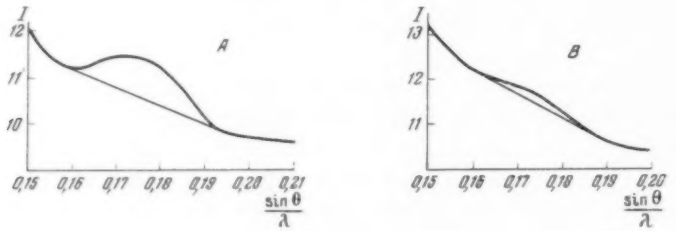

FIG. 1. Maxima for the 2·9Å reflections. *A*: RTT collagen. *B*: spongin.

The integral intensities and excess over background were determined in absolute units for two characteristic spot series: a meridional one with $d = 2{\cdot}9$Å and an equatorial one with $d = 11{\cdot}5$Å.

The intensity distributions for these are shown in Figs. 1 and 2.

The main errors which might enter in the absolute intensity and peak height measurements are due to inaccuracies on the photographic side, and to incorrect extrapolation of the background below the maximum. The errors were estimated by comparing the absolute curves for spongin derived from two series of films (10 in each). The maximum deviation between the mean curves was ± 3 per cent. When the data are compared for specimens giving the same X-ray patterns the errors to some extent balance, particularly as regards peak heights. The overall maximum error did not exceed 5 per cent.

We analysed the relation of integral intensity and peak height to contents of various amino acids. Only imino acids, glycine and alanine showed a regular relation. The results are shown in Figs. 3–5.

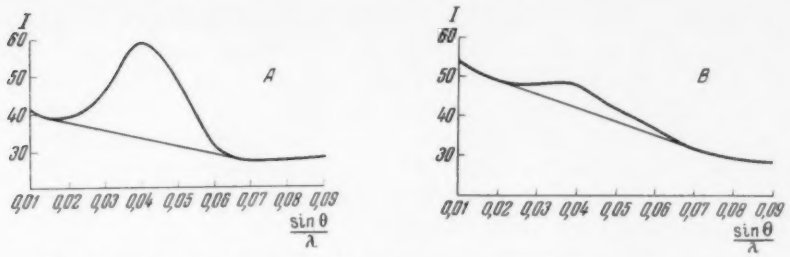

FIG. 2. Maxima for the ~11·5 Å reflections. *A*: RTT collagen. *B*: spongin.

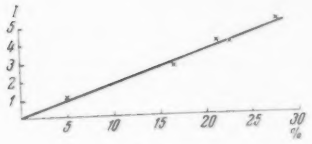

FIG. 3.

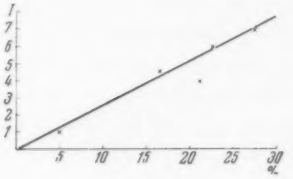

FIG. 4

FIG. 3. Excess over background for the d~11·5 Å series vs. imino acid content in weight per cent.

FIG. 4. Integral intensity for d~2·9 Å reflections vs. imino acid content in weight per cent.

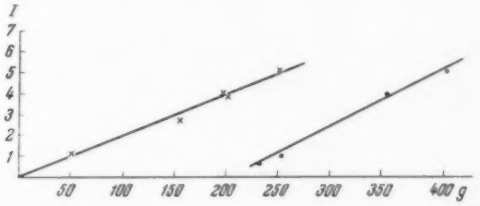

FIG. 5. Maximum height above background for the d~11·5 Å reflections vs. amounts of imino acid and glycine expressed as moles per 10^5 g protein. x: imino acids. ●: glycine.

DISCUSSION

The first striking result is that there are regular linear relationships between integral intensity, peak height and total imino acid content. The straight lines pass through the origin, or very close thereto, allowing for experimental error. The slopes differ for the various series, but the latter may differ on account of specimen anisotropy and distortion. Therefore the differences may not be reliable even as regards different orders from the same plane. This is particularly true of proteins which vary widely in amino acid content, since the chain configuration and packing may be widely variable. But the general changes between specimens indicate changes in the contents of certain structural forms.

The following conclusion can be drawn: the number and size of the areas in the polypeptide chains of collagen having the specific configuration are directly related

to the imino acid content. The nature of the relation indicates that areas with the specific configuration are completely absent at zero imino acid content. Thus the imino acids are necessary and definitive structural elements. The data also imply that the imino acids are almost entirely concentrated in these areas, otherwise the graphs would not pass through the origin. Since even spongin, containing only 5 per cent imino acid, shows the structure, the imino acids must be located in the areas of specific structure. If they were randomly distributed their presence or absence might not in general determine the chain structure.

The relative hydroxyproline and proline contents vary between specimens. Since spongin contains no hydroxyproline the configuration is determined not by its OH group, but by the pyrrolidine ring as a whole. The OH group evidently provides extra stability, thereby giving the various collagens their individual peculiarities [13].

If we combine these data with those on collagen chemistry we can decide which other groups can also determine the chain configuration. These groups must be present in the ratio of one such group per several imino acid residues. About three residues are needed per turn of the collagen spiral: hence the quantitative relationship between the constituent groups of the spiral must be either 1: 1 or 1: 2 if they are of two types; if of three, the ratio is 1: 1: 1. Such fragments can only be of glycine—in which case glycine: proline (or proline: hydroxyproline) ratios of 1: 1 and 1: 2 are possible—of alanine (2: 1) or of polar amino acids. Combinations of imino acid and glycine residues (and also imino acid and alanine) are often observed in partial hydrolysates of collagen: but combinations of these groups with polar amino acid residues are practically never observed [16–18]. There is thus no need to consider these latter.

The integral intensities, the peak heights and the alanine contents show that when the alanine content is zero the total length of areas with the specific chain configuration is still considerable. Hence alanine is not essential to the configuration, so only imino acid and glycine residues determine the collagen spiral (Fig. 5).

The results do not exclude the possibility of other amino acids being present in particular segments of the chain: they only demonstrate that no other groups apart from imino acids and glycine can determine this particular spiral. Obviously, all other residues replace individual groups in the spiral statistically: either altering them or otherwise successfully combining with them.

It is interesting to analyse the latest model of collagen suggested by Ramachandran [19] in the light of our results. He has based his model on a sequence of amino acids of the kind proline–hydroxyproline glycine, permitting the possible replacement of hydroxyproline by other hydroxyamino acids. The molecule is an intricate complex of three interlacing spirals: the large spacing (640 Å)—characteristic of the collagen structure—is explained as a result of the mutual disposition of the three chains in the molecule. The amino acids which compose the sequence chosen by Ramachandran correspond to those which, according to our results, must be situated in areas with the above chain configuration. However, our results seem to point to a 1: 1 relationship, whereas for the Ramachandran sequence the ratio would be 1: 2. The presence of imino acid and glycine accumulations in separate parts of the molecule also supports the hypothesis that regions of different density alternate along the collagen fibrils. In this way it is possible to give a more convincing interpretation of the low-angle

X-ray scattering and of electron microscope data on the collagen fibre using a model with density fluctuations along its length. Ramachandran has suggested an explanation of these facts which depends on the existence of very large regions—several thousand ångstroms long—on the collagen fibril, with a strictly delimited chain configuration.

Moreover, the interference at $d = 2{\cdot}9$ Å must here be due to at least 10^3 layers, but this is contradicted by the spread in reflection, which is far too large (Figs. 1, 2).

Recently, direct chemical evidence of imino acid and glycine accumulations in the collagen molecule has been obtained. Grassman [20] discovered that long peptides (containing about 18–20 residues) produced from gelatine, can be divided into two groups by amino acid composition. To one of these belong peptides containing almost entirely imino acid and glycine residues; to the other belong peptides composed in the main of polar amino acid residues. These latter peptides contain almost no hydroxyproline or proline residues; which is in excellent agreement with our own investigations.

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In conclusion, we wish to take this opportunity of thanking Prof. V. N. Orekhovich and the members of his laboratory for valuable discussions of our results.

CONCLUSIONS

The aim of the experiments was to explain the existence of a special polypeptide chain configuration within the collagen molecule. X-rays were employed to determine the relation between the average lengths of these parts and the amino acid contents in different collagens.

(1) The specific polypeptide chain configuration present within collagen is shown to be due to imino acid and glycine accumulations. The imino acid: glycine residue ratio is approximately 1: 1 in these areas.

(2) These areas can contain other amino acid residues to a small extent, but these play no part in determining the structure.

Translated by J. E. S. BRADLEY

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STRUCTURAL CHANGES IN MYOSIN ON REVERSIBLE AND IRREVERSIBLE INACTIVATION*

CHANGES IN FLOW BIREFRINGENCE, ATPASE ACTIVITY AND SH-GROUP CONTENT FOR MYOSIN SUBJECT TO CERTAIN TYPES OF INACTIVATION

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(*Received* 3 *September* 1956)

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THE relation of structure to ATPase activity in myosin—one of the main contractile proteins of muscle—is of particular interest because the energy liberated by the ATPase is considered by most workers to be used for muscle contraction. Contraction is accompanied by structural changes, as Muralt's [1] data on the fall in muscle anisotropy on contraction indicate. The problem of whether the energy from ATP is used for contraction or relaxation has not yet been solved. The experimental data support both, and hence the relation between structural and functional properties is of great interest.

Muzheev [2] pointed out that possibility of such a relation in 1949. He deduced it from Liubimova and Engelhardt's work [3] which showed that myosin lost its enzyme activity under the same conditions (temperature and pH) as Muralt and Edsall [4, 5] had shown it to lose its flow birefringence; i.e. the long (macromolecular) form was destroyed. Later Needham *et al.* [6] confirmed that the flow birefringence of myosin was lost spontaneously on storing solutions at room temperature, as had earlier been shown by Liubimova and Engelhardt in relation to the enzyme activity. Their data on the way myosin retained its enzyme activity for long periods at 37°C when ATP was added are particularly important, as are those of Needham *et al.* regarding flow birefringence. The data indirectly confirm Muzheev's [2] idea that the energy from ATP is required to maintain the native structure of myosin which is responsible for the enzyme activity.

Subsequently much work was done on the nature of the myosin ATPase activity, and on the changes in flow birefringence produced by various agents, but none was done on the relation between the characteristic structures and functional properties of native myosin. Thus the relation between ATPase activity and presence of free SH groups has been established [7–10]; the SH groups can be blocked, e.g. by heavy metal salts, and restored by adding substances rich in SH groups, such as cysteine or glutathione. The ATPase activity is greatly reduced by blocking and is restored on reactivation. The changes in flow birefringence have been related to the actions of various agents, including those affecting SH groups [11, 12], but these latter do not appear to be real.

As the structural changes occurring in inactivation are complex and far from clear

* *Biofizika*, 2, No. 3, 304 [Reprint Order No. BIO 36].

we studied only those substances known to act either on the flow birefringence, or on the ATPase activity, or on the SH groups. Of the heavy metal salts which block SH groups we selected $CdCl_2$ as being the most thoroughly investigated. As an SH group restorative we used cysteine. Then we used guanidine at concentrations at which it affects the flow birefringence but not the SH groups. We also used sodium monoiodoacetate, which according to Needham [13] bonds to free SH groups but has no effect on the ATPase activity of myosin. Finally, we used a new method to restore the properties of inactivated myosin, namely, reprecipitation with doubly-distilled water followed by solution in 0·5 M KCl.

METHODS

The arrangement for studying flow birefringence was copied from that in Prof. Tsvetkov's laboratory at Leningrad State University. Our equipment—a dynamo-optimeter using an internal rotor—was made from ebonite to prevent myosin inactivation due to uptake of metal ion from the walls. The rotor had a height of 30 mm

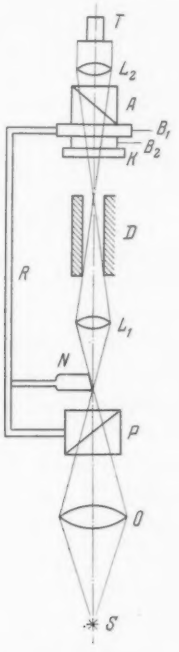

FIG. 1. Optical system.

and an annulus of thickness 0·3 mm. The rotor was driven by two electric motors, the speeds of which could be varied very smoothly with a rheostat device. The rotational speed was read using two stroboscope marks on the motor pulleys, illuminated by neon lamps. The velocity gradient range which could be used while preserving laminar flow ranged from 0 to 1300 sec^{-1}. To measure the birefringence and the indicatrix orientation we found it best to use a half-shadow elliptic compensator.

The optical arrangement is shown in Fig. 1 which is taken from Frisman and Tsvetkov's paper [14].

The polarizer P and analyser A are coupled by the rod R and can be rotated simultaneously in the bearing B_1: between P and A are placed the compensator K (here a 0·034 λ mica plate) and the half-shadow plate N (path difference 0·008 λ) also fixed to R. The compensator K can be rotated relative to A-P in bearing B_2. The light source S (straight incandescent filament) is projected on N by the objective O and then (via the small thin lens L_1) via the gap between the cylinders D. The image of S is viewed in the eye tube T (via lens L_2), the observer seeing simultaneously a bright image of the source, the half-shadow boundary and D_1 which contains the liquid under examination.

The birefringence is determined in the usual way by setting K (rotating in B_2) at the equal brightness position (half-shadow azimuth). The phase difference δ produced by the anisotropic liquid layer was computed from

$$\delta = (\sin 2\eta \pm \sin 2\eta_0) \sin \delta_0$$

where η and η_0 are the half-shadow angles with the inner cylinder moving and at rest and δ_0 is the phase difference introduced by the compensator.

The directions of the principal indices (extinction positions) were determined as follows. With the rotor at rest (birefringence zero) K was set at the half-shadow position. The motor was switched on and the flow birefringence so produced resulted in an intensity difference between the two halves of the field. Rotation of the whole system in B_1 to the appropriate position causes the field to be again uniformly illuminated. This clearly occurred at the extinction position, a principal index coinciding in direction with the vibrational plane of polarizer or analyser. The angular orientation α was determined as half the angle between the two extinction positions corresponding to contrary rotations of the cylinder at equal speeds. This half-shadow method for α almost entirely eliminated errors due to birefringence in the glass ends of the concentric cylinder apparatus or in L_1. The sensitivity was several times higher than without the half-shadow system. The sensitivity can be specified as the least birefringence Δn at which α can be determined with a given accuracy.

The basic experimental quantities in Maxwell's dynamo-optical effect are Δn and α, the angle between the optic axis and the flow direction (the extinction angle). Theory shows that both are single-valued functions of the velocity gradient (g), of the optical properties of the particles, and of some particle-shape function. It is found that α is related to the ratio of length to diameter of the particles; the greater this ratio the less α. The birefringence is the sum of several effects, in addition to the shape factor, including the inherent particle birefringence, the photoelastic and the particle aggregation effects. All the experiments were done with pure myosin, prepared from rabbit muscle by the method of Szent-Györgyi and Liubimova. The protein concentration was determined on each fresh myosin solution by the methods of Kjeldahl and Szent-Györgyi, since the birefringence depends on the protein concentration. Flow birefringence work was always done at a concentration of 1 mg per mm^3.

The ATPase activity was also determined in each experiment by Liubimova's method, SH groups being determined by Mirsky's method, as modified by Tsinirovich and Loseva.

EXPERIMENTAL

The reversible inactivation treatment used was with $CdCl_2$, which blocks SH groups and so depresses the ATPase activity. On adding cysteine, the ATPase activity was restored, though not always completely. When studying the flow bire-

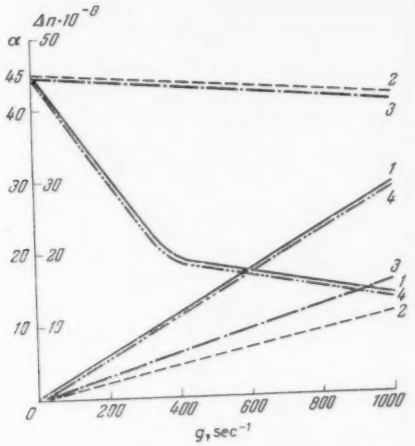

FIG. 2. Δn and α as functions of g for native myosin inactivated by $CdCl_2$ and reactivated by precipitation or by cysteine. (1) normal; (2) $CdCl_2$ inactivated; (3) cysteine reactivated; (4) precipitation reactivated.

fringence as modified by $CdCl_2$ at 2×10^{-5} M it was found that Δn fell sharply as well as the ATPase activity, the depressions on average being equivalent. After adding 10^{-3} M cysteine Δn recovered very slightly, α not being altered at all while on average the ATPase activity was 90 per cent restored.

Complete restoration of the initial Δn, α and ATPase activity was first observed by precipitating the $CdCl_2$ inactivated myosin with double-distilled water, followed by solution in 0·5 M KCl. In Fig. 2 are shown Δn and α for native myosin inactivated with $CdCl_2$ and restored with cysteine, as functions of g.

The free SH group contents were simultaneously measured. The data show (Fig. 3) that the free SH and ATPase activity are depressed together by $CdCl_2$, and are also restored together, at least in the case of water precipitation.

According to Greenstein and Edsall [11] guanidine at low concentrations reduces Δn but not the number of SH groups. The action on the ATPase activity was not checked. We therefore studied the action of guanidine on all three at once. The effect of reprecipitation with water of guanidine-inactivated myosin was also studied. It was found that 0·59 M guanidine reduced Δn and increased α by a factor of three, i.e. acted like $CdCl_2$. The ATPase activity was completely depressed under these conditions. It is of interest that the number of SH groups does not fall under these conditions, but actually rises by more than a factor of two. This indicates denaturation. To a large extent Δn is restored by water precipitation, α less so, and the ATPase activity not at all. The SH group content was reduced, but still exceeded the initial figure. These results are shown in Fig. 4 (Δn and α) and Fig. 5 (the ATPase activity and SH group content).

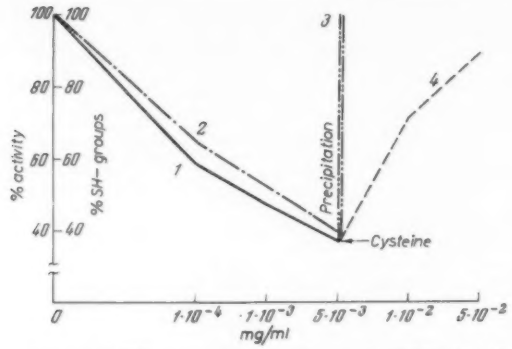

FIG. 3. ATPase activity and SH group content of myosin $CdCl_2$ inactivated and reactivated by precipitation or by cysteine. (1) ATPase activity; (2) SH group content; (3) precipitation reactivated; (4) cysteine reactivated.

The data on the action of monoiodoacetate are also contradictory. Some workers ascribe to it a specific binding to SH groups, thereby considering it to be an inactivator for myosin, which many consider an SH-enzyme. The bonding to free SH groups was demonstrated by Needham. But Liubimova has shown that it has no effect on the enzyme activity. On the other hand Ziff has shown that hydrogen peroxide destroys the enzyme activity of myosin, the activity being restored by cysteine or glutathione. This leads to the conclusion that the SH groups are important to the ATPase activity. But Liubimova has shown from her own and Needham's experiments that we are here dealing with a general change in molecular structure, and not with the simple removal of SH groups. The protein is then not capable of showing its former enzyme activity. In our monoiodoacetate experiments we studied the changes in Δn and α, taking them as indices of molecular shape, in parallel with ATPase activity and SH group determinations.

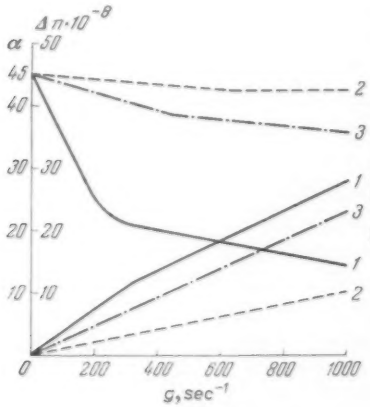

FIG. 4

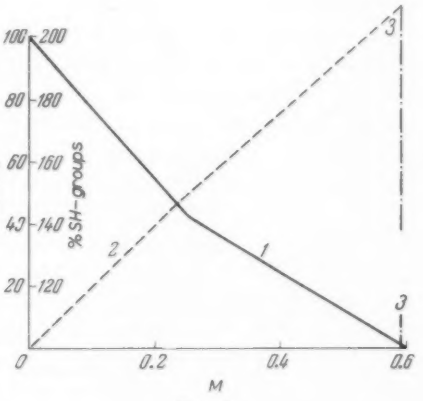

FIG. 5

FIG. 4. Δn and α as functions of g for native myosin inactivated by guanidine and precipitation reactivated. (1) normal; (2) guanidine inactivated; (3) precipitation reactivated.

FIG. 5. ATPase activity and SH group content as affected by guanidine. (1) ATPase activity; (2) SH group content, per cent cysteine; (3) precipitation.

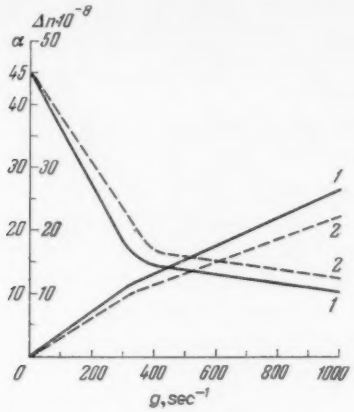

FIG. 6. Δn and α as functions of g for native myosin inactivated by monoiodoacetate. (1) normal; (2) action of monoiodoacetate.

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The experiments show that monoiodoacetate at 1×10^{-4} to 5×10^{3} M gives a small reduction in Δn and a corresponding small rise in α with the native myosin. The data are shown in Fig. 6. The ATPase activity is reduced slightly, on average by 8–10 per cent, and the free SH is also reduced on average by 15–18 per cent as shown in Fig. 7.

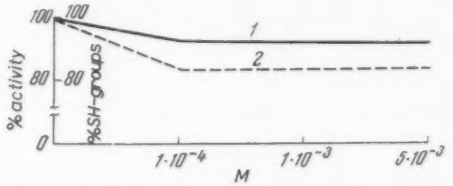

FIG. 7. ATPase activity and SH group content subject to the action of monoiodoacetate. (1) ATPase activity; (2) SH group content.

SUMMARY

The data from the three series of experiments show that all the agents which reduce enzyme activity in myosin also alter the flow birefringence parameters, which indicates that the particle shapes are altered.

The above changes occuring on inactivation usually appear as a fall in Δn and a corresponding increase in α. The data enable us to say that inactivation is accompanied by a rise in molecular symmetry, which can occur either by depolymerization or by coiling. So the drop in enzyme activity accompanies the molecular contraction. The same changes (to a greater or lesser extent) occur with $CdCl_2$, guanidine and monoiodoacetate. But in all three cases the changes in enzyme activity and configuration ran parallel.

The situation is somewhat more complex in $CdCl_2$ inactivation followed by cysteine restoration. Our experiments show that when the enzyme activity is almost completely (90 per cent) restored, Δn is only very slightly restored and α not at all, i.e. the initial particle assymetry is not restored. This is probably related to the fact

that cysteine does not completely restore the activity. The original molecular configuration is probably required for complete restoration. This is confirmed by the water-precipitation experiments with $CdCl_2$-inactivated myosin. All three parameters are here restored completely.

When guanidine-inactivated myosin is restored by precipitation the optical parameters and enzyme activity are together restored slightly. But here, to judge by the free SH group content, the protein is denatured, which prevents any great restoration.

The action of the monoiodoacetate is somewhat peculiar. Our own data on its binding to SH groups differ from Needham's. This is because Needham's nitroprusside method is less accurate than our ferricyanide one for determining SH groups. Our data are shown to be reliable by the complete parallelism between the structural, ATPase and free SH group changes in the monoiodoacetate experiments.

CONCLUSIONS

(1) The loss of ATPase activity in myosin is simultaneous with particle configuration changes.

(2) Cysteine reactivation of $CdCl_2$-inactivated myosin does not restore the flow birefringence parameters.

(3) The enzyme and structural properties of $CdCl_2$-inactivated myosin are completely restored by water precipitation followed by solution in 0·5 M KCl.

(4) The ATPase activity, flow birefringence parameters and free SH groups content change in parallel on inactivating and reactivating myosin.

(5) There is a very close connexion between the structural and enzymatic properties of myosin.

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THE NATURE OF THE CO_2-RECEPTORS IN PHOTOSYNTHESIS*

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(*Received* 14 *September* 1956)

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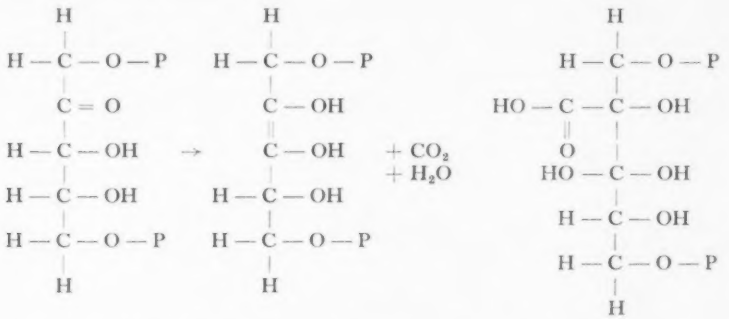
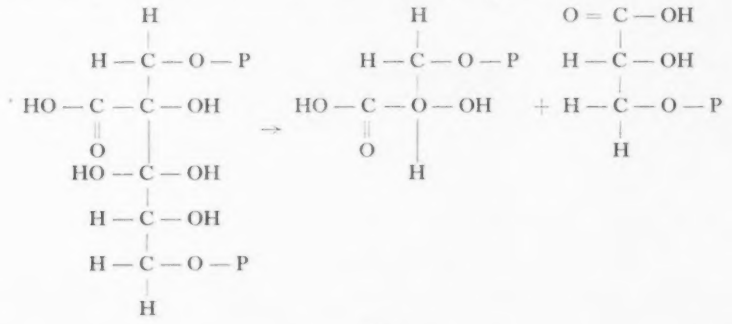
IN 1950 Kuzin and Shkolnik [1] supposed that CO_2 is taken up by substances of carbohydrate type during photosynthesis to give uronic acids as primary photosynthetic products. The uptake by optically active compounds (sugars) was conceivable because only certain of the possible hexose isomers were formed in photosynthesis. Boichenko's work [2, 3] confirmed that the products are uronic acids—he used isolated chloroplasts from higher plants.

Calvin and co-workers [4–6] have supposed that CO_2 is taken up by a hypothetical two-carbon precursor of unknown nature. But in their latest paper Calvin and Bashem [7] conclude from their work on the green algae (*Chlorella*) that under their conditions CO_2 is taken up by ribulose diphosphate giving *iso*hexuronic acid diphosphate as primary product:



this then decomposing to two molecules of phosphoglyceric acid



* *Biofizika*, **2**, No. 3, 313 [Reprint Order No. BIO 37].

Thus the uronic acids were confirmed as the primary photosynthetic products with green algae. But is ribulose diphosphate the only CO_2 acceptor? Ruben and Kamen's work with *Chlorella pyrenoidosa* [8] demonstrated the formation of a carboxyl derivative of molecular weight 1000–1600, which is not diphospho*iso*hexuronic acid (M = 356). Boichenko and Zakharova [9, 10] showed that the CO_2 was taken up by a substance of M about 1500. Doman [11] supposes the CO_2 acceptor linked to a protein.

The present paper deals with the problem whether substances of high molecular weight are the CO_2 acceptors or whether one substance (e.g. ribulose diphosphate) is, or whether various compounds participate, thus giving multiple pathways for photosynthesis. It appeared to us to be important to avoid high temperatures during the separation as these might denature the proteins and alter the primary products if the proteins participate in producing these latter.

Leaves of *Tradescantia quinensis Hort.* were used. The substances formed in the first seconds after initiating photosynthesis were studied. All manipulations were performed at 0°C. The radioactive isotope ^{14}C was used to label the CO_2.

METHODS

The short-duration photosyntheses were performed in the special vessel shown in Fig. 1. The carefully separated leaves, fixed in molybdenum wire loops (since molybdenum does not amalgamate with mercury) were placed in the chamber. The atmosphere in the chamber contained 4 per cent CO_2 by volume, of total activity 82·5 μc (gas volume 68 ml, $^{14}CO_2$ concentration 0·046 per cent). Illumination was by a 500 watt filament lamp 20 cm away. A water filter was placed between the lamp and chamber. The exposures lasted 5 sec.

The leaves (4 g), frozen in liquid nitrogen, were ground with solid CO_2, then

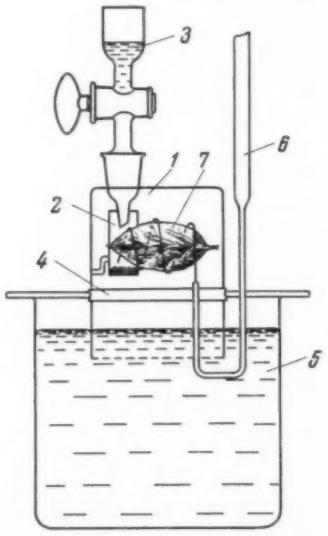


FIG. 1. Photosynthesis chamber. (1) Chamber containing $^{14}CO_2$; (2) small beaker containing $Ba^{14}CO_3$; (3) funnel containing lactic acid; (4) holder; (5) mercury; (6) loop; (7) leaf.

neutralized with Mg $(OH)_2$ at 0°C, and a suspension dialysed at 0°C. The dialysis membranes were prepared from commercial collodion (a 4 per cent solution of nitrocellulose in an ethanol/ether mixture, 1: 3 by volume). The membranes were prepared by pouring the collodion into glass three times (each time pouring off the excess) and the layer was dried in air for 10 min. Dialysis was against distilled water (10–12 ml), changed every 30 min. The activities of suspension and dialysis water were measured.

The dialysable substances were studied via autoradiographs of their chromatograms. The dialysates were concentrated *in vacuo* at 25°C and placed on paper either directly or after fractionation (see below). Fast paper No. 2, for chromatography, prepared by the Leningrad paper works, was used, development being by a butanol/acetic acid/water mixture (40: 10: 50). After drying the chromatograms radioautographs were taken on high-sensitivity X-ray film (XX), using 30–60 day exposures.

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RESULTS

Several experiments showed that the CO_2 acceptors formed completely dialysable compounds. Of the initial activity, 97–98 per cent was found to pass through the membranes in 70 hr.

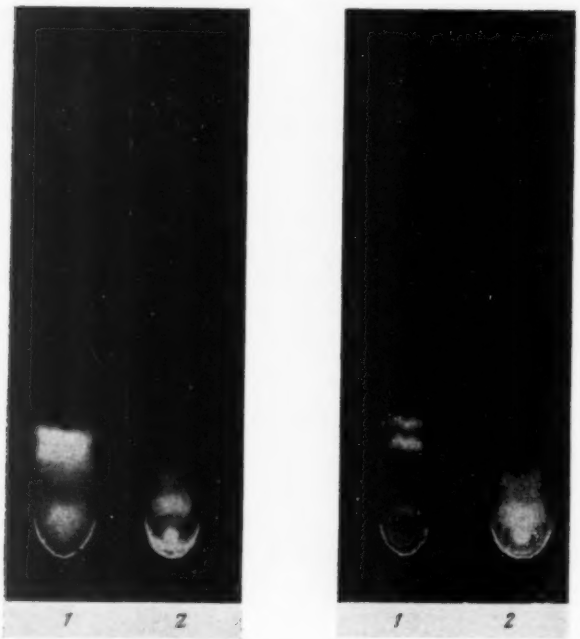
With the aim of retarding photosynthesis we carried out low-temperature photosyntheses in addition to the room-temperature experiments. The mercury in the chamber was cooled to +1°C with snow, dry ice being placed at the top of the chamber. Leaf work-up was as before. Dialysis at 0°C lasted 45 hr. In this period all the activity passed through membrane. (The activities of the photosynthetic products obtained in the low-temperature work were minute and difficult to work with further.)

Most of the active material (74 per cent) passed through the semipermeable membrane in the first 5 hr, half of this passing through in the first hour at 0°C. Supposing that CO_2 might be taken up by substances of widely differing molecular weights, we investigated the first fractions passing through readily, and the later ones, separately.

The dialysates (concentrated at 25°C) were chromatographed. Radioautographs from the chromatograms are shown in Fig. 2. Two substances of principal activity can be seen in the first fraction (R_f 0·06 and 0·05), these being absent from the second the activity being distributed over substances of very low mobility in the latter.

The chromatographic separation was clearer if the solutions were heated to 100°C for 2 min prior to chromatography; this is clear from the radioautographs of Fig. 3.

That there are many CO_2-fixation pathways was demonstrated in another way. The *Tradescantia* leaves were frozen in liquid nitrogen after a 5 sec exposure in the chamber, ground with solid CO_2, neutralized at 0°C with $Mg(OH)_2$ and the material containing ^{14}C separated by dialysis at 0°C for 1·5 hr. The dialysates were concentrated to 8 ml *in vacuo* at 25–27°C and ethanol added to a final concentration of 80 per cent. The precipitate (*A*) could contain the magnesium salts of uronic acids. The filtrate was treated with 80 per cent ethanol saturated with $BaCl_2$. The resulting precipitate (*B*) contained barium salts. The precipitates were dissolved in a small volume of 10 per cent acetic acid and transferred to paper for chromatography. The activities of the parent materials were measured at the same time. It was found that *A* was four times more active than *B*. The radioautographs from the circular chroma-



FIGS. 2 and 3. Radioautographs from chromatograms. (1) Fraction 1; (2) Fraction 2.

tograms are shown in Fig. 4. Three active substances are clearly visible in *A* and four in *B*. The fraction *B* was that in which one of us [1] had earlier demonstrated

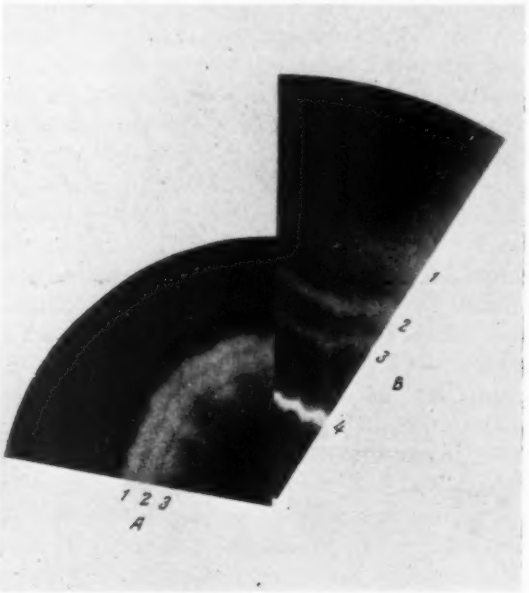


FIG. 4. Radioautographs from circular chromatograms.

a parallelism between uronic acid content and photosynthesis rate. Development of the chromatograms with bromophenol blue showed that some of the active substances were acids, while others (2 and 3 in *B*) were neutral.

CONCLUSIONS

(1) It has been shown that the green leaves of a higher plant (*Tradescantia*) fix CO_2 in compounds which pass through a collodion membrane. Materials of higher molecular weight could not be detected even under mild conditions (0°C), which rules out proteins as primary CO_2 acceptors.

(2) The ^{14}C appears in substances with varying R_f values and salt solubilities within the first 5 sec of photosynthesis. In all, seven substances with different R_f were observed (for *A* the values were 0·36, 0·32, 0·27; for *B* 0·64, 0·47, 0·39 and 0·21).

(3) There is much ^{14}C in the fraction which had earlier been found to show a correlation between uronic acid content and photosynthesis rate.

(4) The data once again indicate a multiplicity of photosynthesis pathways even in the first stages, and are in good agreement with uronic acids being the primary photosynthetic products.

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INFLUENCE OF IONIZING RADIATIONS ON THE SORPTION PROPERTIES OF THE TISSUES OF VARIOUS ORGANS* *IN VIVO*

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(*Received* 6 *November* 1956)

THE study of physico-chemical changes in tissue substrates *in vivo* is of particular interest for understanding the hidden processes which characterize the initial biological effects of ionizing radiations. It seemed to us that any changes set up by ionizing radiations in the high molecular weight substances, which form microstructures in growing tissues or polar groups on micelle surfaces, must be evident

* *Biofizika*, **2**, No. 3, 318 [Reprint Order No. BIO 38].

first of all in the sorption properties of the tissue. The present investigation was based on this assumption.

For studying the sorption properties of living tissue we used radioactive colloids, viz. chromic phosphate labelled with radiophosphorus (^{32}P), iodinated albumin (^{131}I) and colloidal silver solution (^{110}Ag). Moore [1] was the first, in 1942–43, to use radioactive colloids, namely, brominated dyes (^{82}Br) for studying the adsorptivities of various tissues. In 1944 Jones [2] described a method of preparing a phosphorus-labelled colloidal chromic phosphate for studying the absorbing power of the reticuloendothelial system (RES). In 1949 Dobson [3] studied the absorption of colloidal zirconium, riobium, yttrium and lanthanum salts as a function of the particle size. Brownell prepared colloidal radioactive silver [4] and also used it for investigating the absorbing power of various tissues. Similar studies were undertaken by other authors [5–9]. Since 1951 investigators have been endeavouring to use radioactive colloids for studying the effects of ionizing radiations.

Barrow [10], using colloidal gold, found that the sorptive power of the RES of rabbits remained unaffected by irradiation with doses of 500–800 r. Esplin [11], using thorium oxide, came to a similar conclusion. In 1953–54 Gabrieli [12] published the results of his tests with radioactive chromic phosphate. He succeeded in measuring changes in the absorption of colloids by the liver, and observed increases in the total capacities of livers and spleens of rats irradiated with doses of 25, 50 and 100 r.

The published data are contradictory. We thought it worth while, therefore, to study the sorption properties of tissue and how they are affected by ionizing radiations. A number of different radioactive colloids were used to preclude the effects of particle size observed by various authors. A comparison of the data *in vivo* (particularly on the RES) with those on perfused liver tissue (to exclude the effects of circulatory changes) were also made. Results of the tests carried out in 1954–55 are given below.

METHODS

The radioactive chromic phosphate was prepared by Jones's method [2], the colloidal solution of iodinated (^{131}I) serum globulin by Francis's method [13] and the solution of colloidal silver (^{110}Ag) with protective gelatin by Brownell's method [4].

An electron microscope was used for determining the particle size and its uniformity. Chromic phosphate prepared by Jones's method proved to be of very irregular size, varying between 0·001 and 1μ. Samples of the colloidal silver, as shown in Fig. 1, showed a very uniform particle size of between 0·06 and 0·001μ.

White male rats weighing 120–130 g were used for the tissue absorption tests *in vivo* and for studying the effect of radiation on them. A volume of 1·0–1·5 ml of colloidal solution, having an activity of about 0·5 μc, was infused into the femoral veins of rats during 10 min. Two hours later the animals were decapitated (by which time the blood activity was zero). The isolated organs were dehydrated three times with alcohol (96 per cent), which was then centrifuged off and the tissue washed twice with ether. The tissue insoluble in alcohol and ether was dried in a vacuum desiccator. It was found that all the chromic phosphate and iodinated albumin activities remained in the residue; some of the colloidal silver was removed by the lipoid solvent, and this induced us to test also tissue air-dried at 80°C. The activities

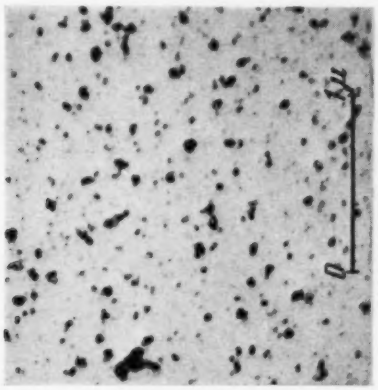

FIG. 1. Electron-micrograph of colloidal silver solution.

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of the dried tissue specimens (10 mg/cm^2), were determined at constant geometry with a B-2 tube (for ^{32}P) and with a scintillation counter (for ^{131}I and ^{110}Ag). The rate of absorption of the radioactive colloid by the liver was also determined *in vivo* by means of a scintillation counter inserted in the opened abdominal cavity. In these tests the counter crystal was placed directly above the bare liver so as to permit observation of the changes of hepatic surface activity after injecting radioactive chromic phosphate into the femoral vein.

The sorption capacity of the perfused liver was studied by means of a radioactive colloidal silver solution (^{110}Ag).

Rats weighing 230–250 g were used for the liver perfusion tests, under urethane narcosis with heparinization to prevent cardiac clogging. The peritoneal cavity was opened 5–10 min after injecting heparin and Ringer's solution was introduced into the portal vein through a cannula. The thoracic cavity was then exposed and a second cannula introduced for drainage into the inferior vena cava (Fig. 2). The infused solution was saturated with oxygen and kept at a constant temperature of 37°C. The inlet pressure was 13–14 mm Hg. (The liver did not swell under these conditions.) The equipment used enabled us to replace Ringer's solution by the test solution which entered the liver at the same pressure and temperature. Both the Ringer's solution and the test solution of radioactive colloidal silver in Ringer's solution had a pH of 7·4. The effluent flowed at a constant rate through a special flat glass chamber, the ^{110}Ag activity being measured by a scintillation counter. The colloid was passed until the liver was completely saturated, the activity of the outgoing liquid being constant and equal to that of the incoming liquid. The time-course of absorption was measured. After saturation the liver was washed with Ringer's solution, to remove any unabsorbed radioactive silver, and fixed; part of the liver was then examined for its colloid content by the method already described.

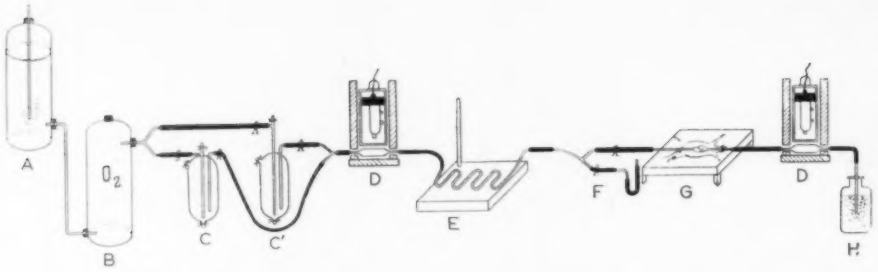

FIG. 2. Diagram of arrangement for liver perfusion *in situ*. A—water container; B—oxygen container; C—Mariotte flask with Ringer's solution; C¹—Mariotte flask with colloidal silver solution; D—scintillation counters for recording the activities of incoming and outgoing fluids; E—bath for maintaining the temperature; F—mercury manometer; G—liver isolated *in situ*; H—container for discharged fluid.

A three-tube X-ray unit was used to irradiate the rats at 170 kV, 30 mA, with 0·5 mm Cu + 0·75 mm Al filters, dose-rate 43 r/min. All experiments were performed after giving 1000 r. Measurements were made 2, 24 and 48 hr after irradiation.

RESULTS

Preliminary tests showed that radioactive chromic phosphate and iodinated γ-globulin introduced into the femoral vein are rapidly absorbed by the internal organs. The rates at which iodinated γ-globulin was eliminated from the blood of control and irradiated rats is given in Fig. 3 which shows that the colloid practically disappears from the blood in 60–75 min. The increase in liver chromic phosphate activity *in vivo*, as measured with the scintillation counter, is shown in Fig. 4. It will be seen that activity ceases to increase after 8–10 min. Preliminary tests with irradiated animals show that the rate of absorption of the colloid tends to fall at 24 hr after irradiation.

The question of which organs showed changes in absorption also seemed worth investigating and we accordingly measured the absorption of the colloids in the liver, spleen, lungs, kidneys, small intestine, spinal cord, brain and muscular tissue.

Table 1 shows the distribution of radioactive colloids in the tissues of control animals as measured from three tests which were in good agreement for each colloid. The bulk of the colloid is absorbed by the liver. The relative sorption of a colloid by the tissues of different organs varies within narrow limits. The thyroid gland is particularly active in the case of iodinated proteins, which seems to indicate that the iodine is partially split off in the organism; this would also explain the somewhat greater relative activity of muscles in the iodinated globulin tests. The colloid distributions in the irradiated organs were measured 2, 24 and 48 hr after irradiation (1000 r). The data on the irradiated animals are compared with those for the controls (non-irradiated) in Table 2.

All the organs investigated showed less pronounced changes of sorption properties than the liver; particular attention was therefore given to this organ in the subsequent tests, the changes of its sorption properties being measured at different times after irradiation.

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TABLE 1. RELATIVE ABSORPTION OF COLLOIDS BY THE TISSUES OF VARIOUS ORGANS

Tissue tested	Mean weight of organ (g)†	Chromic-phosphate (66,238 counts/min in rat)*				Iodinated globulin (180,400 counts/min in rat)			Colloidal silver (63,140 counts/min in rat)		
		Dry: Moist weight ratio	Activity of dried tissue (counts/min per 10 mg)	Distribution of colloid (counts/min in whole organ)	Distribution of colloid in tissue (per cent of activity injected)	Activity of dried tissue (counts/min per 10 mg)	Distribution of colloid (counts/min in whole organ)	Distribution of colloid in tissue (per cent of activity injected)	Activity of dried tissue (counts/min per 10 mg)	Distribution of colloid (counts/min in whole organ)	Distribution of colloid in tissue (per cent of activity injected)
Liver	8·4	0·15	413±15	52,038	78±2·8	859±37	108,234	60·0±2·2	363±20	45,738	72±0·4
Spleen	0·4	0·2	65±5	520	0·78±0·06	413±18	3304	1·8±0·07	66±4	528	0·8±0·05
Lungs	0·9	0·14	60±10	756	1·10±0·19	41±7	517	0·28±0·04	18±3	226	0·35±0·06
Kidneys	1·3	0·14	10±3	182	0·27±0·08	89±10	1620	0·89±0·1	12±2	218	0·34±0·05
Small intestine	5·3	0·13	7±2	483	0·72±0·2	15±3	1035	0·5±0·1	10±2	690	1·0±0·2
Spinal cord	0·4	0·14	5±2	28	0·04±0·01	0	0	—	5±1	28	0·04±0·008
Brain	1·8	0·19	5±1	171	0·25±0·05	0	0	—	6±1	205	1·10±0·05
Muscles	44	0·14	5±1	3080	4·6±0·9	25±5	15,400	7·1±1·7	3±1	1848	2·9±0·9
Thyroid gland	0·03	0·14	—	—	—	20,500±52	8610±21	4·6±0·01	—	—	—

* The relative activities are given in counts/min, identical counting conditions being used with all specimens.
† Mean weights of organs according to Donaldson.

TABLE 2. DISTRIBUTION OF COLLOIDS IN THE TISSUES OF VARIOUS ORGANS AS PER CENT OF ACTIVITY INJECTED

Tissue tested	Chromic phosphate				Iodinated albumin					Colloidal silver			
	Controls	2 hr	24 hr	48 hr	Controls	2 hr	24 hr	48 hr	75 hr	Controls	2 hr	24 hr	48 hr
	Number of animals used												
	3	4	5	4	3	4	5	4	4	3	4	5	5
Liver	78 ± 2·8	72 ± 2	56 ± 1·5	44 ± 1·8	60 ± 2·2	58 ± 1·9	46·5 ± 1·6	48 ± 2·0	46 ± 2·1	72 ± 0·4	70 ± 0·3	52 ± 0·2	64 ± 0·2
Spleen	0·78 ± 0·06	1·1 ± 0·07	0·7 ± 0·06	0·38 ± 0·05	1·8 ± 0·07	1·9 ± 0·08	1·4 ± 0·09	1·0 ± 0·02	1·2 ± 0·09	0·8 ± 0·05	1 ± 0·05	0·9 ± 0·04	0·9 ± 0·04
Lungs	1·1 ± 0·19	1·1 ± 0·13	1·0 ± 0·11	1·3 ± 0·15	0·28 ± 0·04	0·29 ± 0·04	0·26 ± 0·05	0·23 ± 0·08	0·21 ± 0·03	0·35 ± 0·06	0·34 ± 0·07	0·32 ± 0·08	0·35 ± 0·07
Kidneys	0·27 ± 0·08	0·29 ± 0·09	0·26 ± 0·08	0·30 ± 0·06	0·89 ± 0·1	0·90 ± 0·1	0·91 ± 0·1	0·88 ± 0·1	0·8 ± 0·1	0·34 ± 0·05	0·35 ± 0·06	0·36 ± 0·07	0·34 ± 0·06
Small intestine	0·72 ± 0·2	0·58 ± 0·1	0	0	0·5 ± 0·1	0·4 ± 0·09	0	0	0	1·0 ± 0·2	0	0	0
Spinal cord	0·4 ± 0·01	0·03 ± 0·01	0	0	0	0	0	0	0	0·04 ± 0·08	0	0	0
Brain	0·25 ± 0·05	0·21 ± 0·04	0	0	0	0	0	0	0	1·1 ± 0·05	0·8 ± 0·04	1·0 ± 0·05	0·9 ± 0·04
Muscles	4·6 ± 0·9	4·6 ± 0·8	4·5 ± 0·9	4·5 ± 0·9	7·1 ± 1·6	7·8 ± 1·6	8·1 ± 1·5	8·1 ± 1·7	8 ± 1·7	2·9 ± 0·9	2·9 ± 0·9	3·0 ± 0·8	3·2 ± 0·7
Thyroid gland	—	—	—	—	4·6 ± 0·01	4·4 ± 0·01	4·6 ± 0·02	4·8 ± 0·01	4·9 ± 0·01	—	—	—	—

Note: The times given in the table are those elapsed after irradiation.

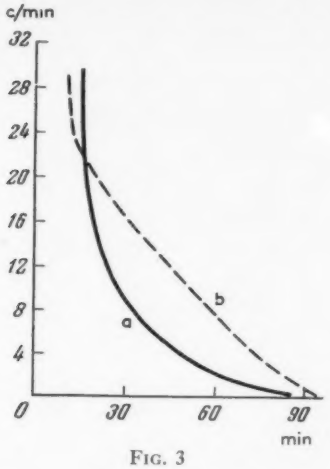

FIG. 3

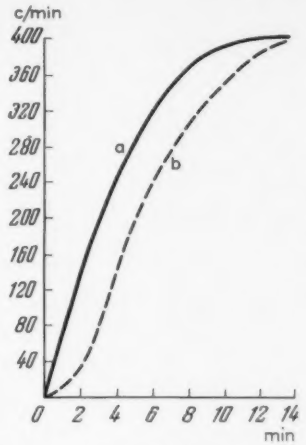

FIG. 4

FIG. 3. Elimination of labelled albumin from the blood of a rat. a—no irradiation; b—24 hr after irradiation.

FIG. 4. Absorption of chromic phosphate suspension by rat liver. a—liver not irradiated; b—liver 24 hr after irradiation.

As shown in Table 3, the sorption capacity of hepatic tissue tends to decrease within 2 hr of irradiation. The decrease is much more pronounced after 24 hr, amounting to 25–30 per cent for all the colloids tested. At later periods after irradiation the absorbing power of hepatic tissue varies according to the colloid used. Thus, for example, it continues to decrease after 48 hr in the case of a coarse suspension of chromic phosphate, but it increases somewhat for highly disperse colloidal silver and tends to become normal.

TABLE 3. CHANGES IN ABSORBING POWER OF HEPATIC TISSUE AFTER IRRADIATION, AS PER CENT OF CONTROLS

Colloid	Time after irradiation			
	Controls	2 hr	24 hr	48 hr
Chromic phosphate	100	92	71	56
Iodinated globulin	100	86	76	80
Colloidal silver	100	97	72	88

Introduction of small quantities of colloids *in vivo* failed to produce saturation of the tissue and the curves obtained result not only from changes in absorbing power of the tissues, but also possibly from disturbances in the circulation. The sorption capacities of perfused isolated livers were studied by the method already described. We also examined the isolated livers of control and irradiated rats, isolating those of the latter 2, 24 and 48 hr after whole-body irradiation (1000 r). The uptake curves for the radioactive colloids are shown in Fig. 5.

Analysis of the activity curves of the outgoing solution shows that total saturation of perfused livers from irradiated animals is reached more slowly than in the controls.

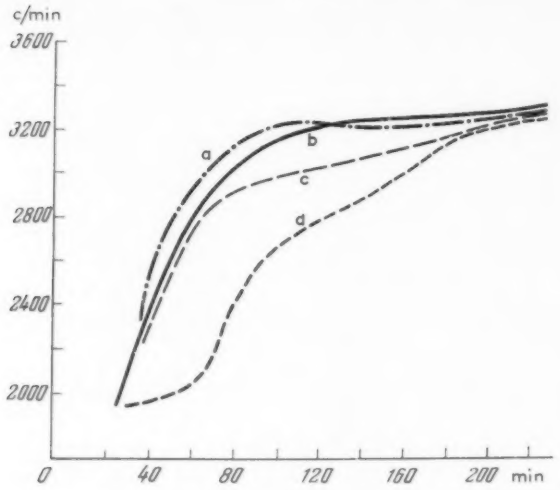

FIG. 5. The uptake curves for colloidal silver by isolated rat liver. a—liver not irradiated; b—liver 2 hr after irradiation; c—liver 24 hr after irradiation; d—liver 48 hr after irradiation.

To a slight extent this slower rate becomes evident within 2 hr after irradiation and is very pronounced after 24 hr.

The sorption capacity of the liver was measured both by the activity of the liquid flowing through it and by direct determination of the tissue activity. The latter was measured after saturating the liver with the radioactive colloid and subsequent washing with Ringer's solution. The results are given in Table 4.

TABLE 4. ABSORBING POWER OF ISOLATED LIVER

Test no.	Time after irradiation (hr)	Determined from difference of activity between incoming and outgoing liquid (per cent of controls)	Determined from activity of air-dried hepatic tissue (per cent of controls)
1	Controls	100	100
	2	87	85
	24	60	63·5
	48	90	95
2	Controls	100	100
	2	84	94
	24	48	60
	48	78	86
3	Controls	100	100
	2	94	91
	24	53	58
	48	78	89

The absorbing power of the liver, as determined both from the activity of the perfusing liquid and from that of the dried tissue, begins to decrease within 2 hr

after irradiation, falls by 30–40 per cent within 24 hr and increases again 48 hr after irradiation.

DISCUSSION

The results show that the bulk (about 70 per cent) of intravenously injected radioactive colloid is absorbed by the liver in rats. A considerably smaller quantity of the radioactive colloid is found in the spleen, lungs and small intestine. Practically no colloid is found in the brain. The injected colloids are eliminated from the circulation more slowly at 24 hr after irradiation. The rates of elimination of the colloids from the circulation are not, however, criteria for changes in tissue absorptivity, still less for the absorptivities of the organs. This question can be answered only by quantitative measurements of the colloids absorbed by the tissues.

Barrow's conclusions as to the radioresistance of the RES took no account of the activities of the various organs and therefore appear to us to be premature.

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Our tests show that after X-ray irradiation with 1000 r the absorptivities of the livers of rats are appreciably lowered within 2 hr after irradiation, while within 24 hr they decrease by 25–30 per cent. These data agree with those of Luzio [14] who found that a dose of 1000 r reduces by 30 per cent the quantity of radioactive colloidal gold absorbed by rat liver. He attributes this reduction to disturbance of the circulation and increased capillary permeability.

Our similar data were obtained by liver perfusion which precludes disturbance of the circulation and this factor cannot therefore account for the reduction observed. Limperos and Mosher [15], Kuzin and Budilova [16], Edgerley [17], Kiselev [18] and others have shown that, during the first 24 hr after irradiation, radiosensitive tissue shows considerable depolymerization of high polymers, e.g. nucleo-proteins, nucleic acids, glycoproteins, mucopolysaccharides, etc. Depolymerization inevitably involves physico-chemical changes in the protoplasm of radiosensitive cells, in particular as regards sorption properties and capillary permeability which apparently reduce the absorptivity of the tissue.

Absorption of inert colloids circulating in hepatic blood vessels and capillaries, occurs apparently both in the endothelial cells lining these vessels and capillaries, and in the Kupffer cells of the RES. The changes here described in the sorption properties of the liver during the first 24 hr after irradiation seem to indicate that those cells which participate in colloid absorption are quite radiosensitive. Depolymerization of high polymers apparently modifies the physico-chemical properties of the cell protoplasm which, in turn, disturbs adsorption of the colloids and absorption by the protoplasm (phagocytosis). These changes are quite comparable with the reduction in antibody synthesis in the irradiated organism, which apparently is closely linked with the observed changes of sorption properties in the cells of the RES.

The subsequent increase (48 and 75 hr after irradiation) of adsorptivity of the liver is apparently connected with subsequent polymerization of the macromolecules depolymerized during the previous 24 hr. It is not clear whether this produces the original structures or whether the changes occurring in the molecular structures during the first 24 days give rise to the abnormal structures; these problems call for further investigation.

Radioactive colloidal silver, finely dispersed and of constant particle size, proved the most suitable radioactive colloid for our purposes. The disadvantage of chromic

phosphate (used by various investigators [2, 12]) is its relatively large and irregular particle size. Iodinated albumen has some of its iodine split off in ionic form in the organism and this renders evaluation of its real distribution difficult.

CONCLUSIONS

The sorption capacities of tissues (rats) were investigated *in vivo* 2, 24 and 48 hr after X-ray irradiation (1000 r) using radioactive colloidal chromic phosphate (^{32}P), iodinated globulin (^{131}I) and a colloidal silver solution (^{110}Ag).

It is shown that about 70 per cent of the colloid injected was absorbed by the liver. Within 2 hr of irradiation, and more particularly 48 hr later, the absorptivity of the liver decreased. From 48 to 75 hr the sorption capacity of the liver again increases.

It is suggested that this reduction may be due to physico-chemical changes in the macromolecules which form the surfaces and protoplasm of cells in the reticuloendothelial system, and that it is closely linked with changes in antibody synthesis.

Translated by O. S. WHITSTON

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THE QUASI-CONSTANT POTENTIAL IN DIFFERENT LEVELS IN THE CEREBRAL CORTEX AND ITS INFRA-SLOW RHYTHMIC CHANGES STUDIED USING A MICROELECTRODE TECHNIQUE*†

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(*Received* 19 *October* 1956)

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Every point on the cortical hemisphere has some electrical potential relative to other regions or to indifferent points distributed over the cranium. The magnitudes of these potentials are altered considerably when the functional state of the cortex is disturbed. For example, in ischaemia of the brain and asphyxia [1, 2] the potential of the cortex changes by some millivolts and becomes more negative relative to the indifferent regions. Stimuli applied to the cortex (chemical, electrical or mechanical) cause the brain surface potential to become more negative [2], the change spreading over the surface and into the underlying layers. This potential is not strictly constant, but changes slowly; hence we term it the quasi-constant potential.

It has been found that a relationship exists between the nature of the impulse activity of the cortical neurones and the size and sign of the quasi-constant potential in certain regions of the cortex [3, 4]. Thus, after an increase in the negative potential a depression in the "spontaneous" electrical activity on the electroencephalogram (EEG) is seen [2, 3]. The neuronal excitability of the cortex is raised when the caudal electrode is made positive [3].

Previously Aladzhalova [3] had discovered that the quasi-constant potential of the cortex undergoes an ultra-slow rhythmic change with a period of 8–10 sec (secondary rhythm) and 0·5–2 min (minute rhythm). The corresponding frequencies of these rhythms are 6–8 and 0·5–2 cycles per min, respectively. They are termed ultra-slow to distinguish them from rhythms, the periods of which are in the region 0·02–0·5 sec or less.

It has been made clear [3, 5] that the amplitude and frequency of the ultra-slow rhythmic change can be altered by a number of influences; by metabolic disturbance, afferent stimulation and development of intra-cortical irritation. In addition, the excitability of certain parts of the cortex is changed with the appearance of ultra-slow changes of potential and "spontaneous" activity is shown [3].

Fluctuating patterns on the electroencephalograph emerge very clearly at different phase values of the ultra-slow waves by the appearance of a series of high-amplitude discharges [5]. On these occasions burst discharges occur which are synchronized with the maxima of the ultra-slow waves of quasi-constant potential, i.e. during the time when the more caudal parts of the cortex are made positive.

† Read in the Department of Biophysics, Lomonosov State University, Moscow, 21 May 1956.

* *Biofizika*, **2**, No. 3, 327 [Reprint Order No. BIO 39].

It has been reported that constant potential is found not only over the surface of the cortex but also in deeper levels [2]. For example, there is a definite difference of potential between the surface of the cortex and the ventricle, the visual cortex having a relative superficial charge of +5·5 mV to —1·7 mV [6].

The presence of such gradients of electrical polarity makes it possible to assume the existence of currents between the different strata of the cortex, caused by inequalities in the distribution of electrolytes between extracellular fluids. An understanding of the mode of origin of these differences in potential between different parts of the cerebral cortex, and their functional significance, required a detailed study of the distribution of the quasi-constant potential among different structural levels in the cortex.

METHODS

Gentle ether narcosis was followed by trepanning of the skull of the rabbit above the sensimotor region of one hemisphere. To immobilize the animal Diplatsin, a preparation of *d*-tubocurarine, was used with artificial respiration. The tough cerebral membrane was removed during the trepanning. For the recording a non-polarizing microelectrode was used, of diameter 8–12 μ and a d.c. resistance of 300 kΩ to 2 MΩ. Microcapillaries filled with physiological saline were used in conjunction with the non-polarizable system. Insertion of the microelectrode to different levels of the cerebral cortex was carried out with the microelectrode perpendicular to the surface of the hemisphere. Contact of the electrode with the cortical surface, after the removal of excess fluid, was indicated by a jump of potential. Dipolar recordings were made with two microelectrodes on different parts of the cortex. Unipolar recordings, and on later occasions, recordings with indifferent electrodes of silver plate arranged symmetrically on the hemisphere were also made. In the course of a series of experiments the positions where the electrodes were placed underwent thermocoagulation. Recording of the potential wa seffected by d.c. amplification and direct coupling to a recording oscillograph [3]. Zero drift during amplification was virtually absent.

RESULTS

In the course of a series of experiments it was found that the size of the electrical potential changed during insertion of the electrode (unipolar recording) into different levels of the cortex. The initial differences in the potentials of active microelectrodes, placed on the surface of the cortex, were compensated by a circuit for amplification and adjustment to zero. Thus, the difference in potential at different levels in the cortex was compared by us with the potential of the surface of the hemisphere.

It was established that when a microelectrode was inserted to definite depths into the cortex, the potentials recorded were all more negative than that at the surface. During further insertion into the cortex, to spaced depths, the size of the potential began to change in the opposite direction; negative potential being decreased. When the white matter was reached the potential assumed a positive sign relative to the surface of the cortex.

The levels in the cortex through which the microelectrode was passed were mostly of negative potential (Fig. 1). In different experiments the condition of these levels underwent a shift in the region 1·2–1·6 mm, while the size of the negative potential lay within the limits 10–20 mV. During the introduction of a microelectrode into

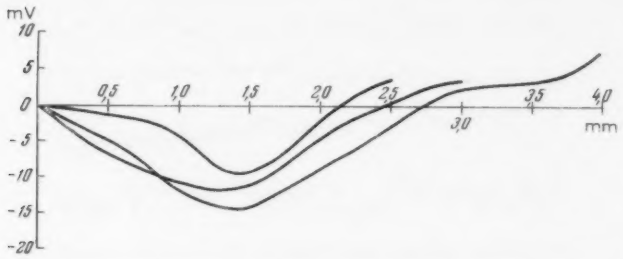

FIG. 1. The potential distribution at different levels in the cerebral cortex of the rabbit (three experiments). The ordinate is the potential differences between the surface of the cortex and the different levels to which the microelectrodes were inserted. The abscissa is the approximate depths of the microelectrode. Beyond 2 mm white matter was encountered.

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the levels of the central convoluted part, the maximum potential was registered at a depth 0·2–0·4 mm deeper than during frontal introduction. To some extent the difference in thickness of the cortex is responsible in the second case. The highest levels of negative potential were recorded in the cortical levels V–VI. White matter was found to have a potential positive to that of the cortex, with the value of 1–5 mV (at depths greater than 2–2·5 mm). This agrees with data of Goldring and O'Leary [6].

Unsuccessful attempts were made to detect areas of high negative potential during deep ether narcosis and death of the animal. Luminal-induced sleep acted similarly on the distribution of the quasi-constant potential at different depths in the cortex. Regions with high negative potential began to lose charge, and there is a drop in the potential gradient between the surface of the cortex and the deeper levels. It is therefore possible to change the distribution of the quasi-constant potential in the deeper layers of the cortex by a number of influences.

Experiments were performed on 12 rabbits and each was repeated several times. Regions of high negative potential were found in all experiments, and always in lower levels of the cortex. There was some degree of spread of the potentials within the limits $\pm$0·25 mm. During repeated introduction of a microelectrode into one and the same region of the cortex, the zone negativity was recorded not at one depth only, but within varied limits of $\pm$0·1–0·15 mm.

In the course of a second series of experiments we were interested to find that the potential of the deep-lying parts of the cortex underwent ultra-slow rhythmic fluctuations.

Unipolar recordings were made during the insertion of a microelectrode to different levels of the cortex, in the same way as for the dipolar recordings. In the second case two microelectrodes were arranged, either at the same depth or at different depths in the cortex. In Fig. 2 is shown the appearance of the ultra-slow waves recorded at different levels in the cortex. Recording *A* was made with a circlet of silver electrodes on the surface of the motor region of the cortex, while recording *B* is a unipolar recording on the surface of the hemisphere with one of the non-polarizing microelectrodes. In both these recordings secondary rhythm is visible, having the frequency of 6–7 c/min. Diagram *C* shows a dipolar recording of the potential between points on the surface of the cortex and at a depth of 0·7 mm. In

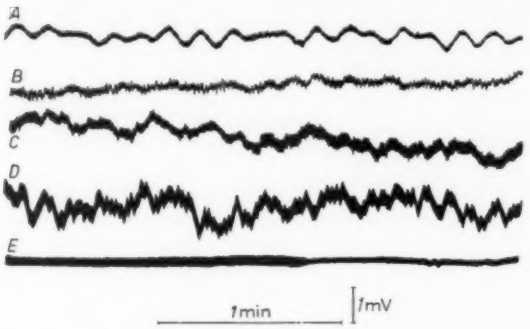

Fig. 2. The ultra-slow rhythmic fluctuations in the quasi-constant potential during different conditions of recording with non-polarizable microelectrodes. *A*: a control recording with permanently attached silver plate electrodes placed on the motor area. *B*: a unipolar recording with the microelectrode on the surface of the cortex. A secondary rhythm is shown with a frequency of 6–7 min^{-1}. *C*: a secondary rhythm of frequency 4–6 min^{-1} picked up by one electrode inserted to 0·7 mm, the other being on the surface. *D*: a wave of frequency 8 min^{-1} picked up by one electrode inserted to 1·5 mm, the other being at the surface. *E*: disappearance of the ultra-slow rhythms during ether narcosis.

these recordings it is possible to distinguish on rare occasions a wave of 4–5 c/min in addition to that of 7 c/min. An example of this is shown in *D*, which is a dipolar recording of the potential between the surface of the cortex and loci at depth 1·5 mm. The disappearance of the ultra-slow rhythm during ether narcosis is shown in *E*. The frequency of the hatchure on the line of the recordings (40–60 per min) is revealed as an artefact of the mechanical pulsation of the brain during respiration.

These data show that the ultra-slow rhythm can be recorded with a non-polarizing microelectrode, and that the rhythm is not confined to the surface of the cortex but extends into the underlying layers.

Detailed study revealed that it is possible to distinguish different amplitudes and frequencies of the rhythm at different cortical levels. This was established by experiments in which two electrodes were placed at one level for one recording and then at another level for a second recording; in one instance, both on the surface of the cortex at a distance of 1·5 mm from one another, and then at a depth of 1 mm, the same distance apart. Comparison of the recordings showed that when the electrodes were on the surface of the cortex, a secondary rhythm of frequency 7 c/min was seen, having an amplitude of 0·9 mV. At a depth of 1 mm a rhythm was registered which was transitory between secondary and minute, having a frequency of 4 c/min and amplitude of —1·5 mV.

In the deeper levels extending to the white matter of the brain and even into the white matter itself, it was still possible to find ultra-slow rhythmic changes in the quasi-constant potential. For example, in Fig. 3 is shown a dipolar recording of rhythmic waves with combined secondary and minute rhythms, which was made with the microelectrode at a depth of 1·7–2·7 mm into the cortex.

In the majority of cases (from 12 to 18) the frequency of the ultra-slow waves was higher in the upper parts of the cortex than in the lower. In Fig. 4 are shown recordings made during the insertion of a microelectrode (unipolar recording) to successive positions in different levels. On tracing *A* and *B* made at depth 0·5 and

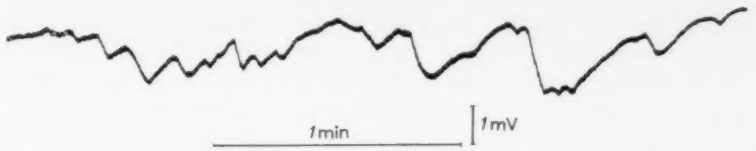

FIG. 3. The superposition of a secondary rhythm (minute interval shown) from deep-seated layers. Dipolar recording at depth 1·7–2·7 mm.

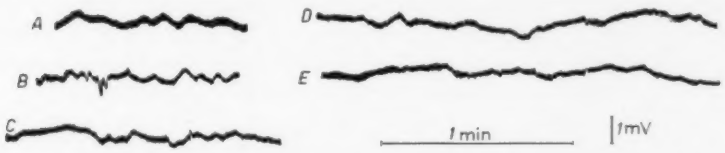

FIG. 4. The rhythmic variations of the potential recorded after insertion of a microelectrode to different depths in the cortex. Unipolar recording. *A*, *B*: secondary rhythm at a depth of 0·5 and 1·0 mm. *C*: secondary rhythm at a depth of 1·5 mm. *D*, *E*: secondary rhythm at a depth of 2·0 and 2·5 mm. A minute interval is shown.

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1 mm is seen only secondary rhythm with frequency 6–7 c/min. At a depth of 1·5 mm, in addition to the secondary, appears minute (*C*), and during further insertion of the microelectrode to a depth of 2 and 2·5 mm only minute rhythm (*D* and *E*) with frequency 1–2 c/min. In these regions of the cortex the zones of greatest negative quasi-constant potential terminate at level 1·5 mm. Thus, it is found that the uppermost levels have definite waves of negative potential with secondary rhythm while the lower levels in the cortex have minute rhythm.

In order to be sure that this distinct picture of the rhythms at the surface of the cortex and at deeper levels is not affected by movement (insertion and withdrawal) of the microelectrode, we used a one-step insertion of the non-polarizing microelectrode, the recordings from which appear in Fig. 5. After the sensimotor region of the left hemisphere was revealed by trepanning, three microelectrodes were inserted; one on the surface of the cortex, the second at depth 1·4 mm and the last at depth 2·4 mm. Recording *A* showing rhythmic waves almost without feature, was made with the upper and middle electrodes. Recording *B* showing ultra-slow waves with a rhythm of 4 c/min was made with the middle and lower electrodes.

In a series of experiments it was found that the ultra-slow rhythms at all levels of the cortex and white matter had equal frequency. Some instability was often seen

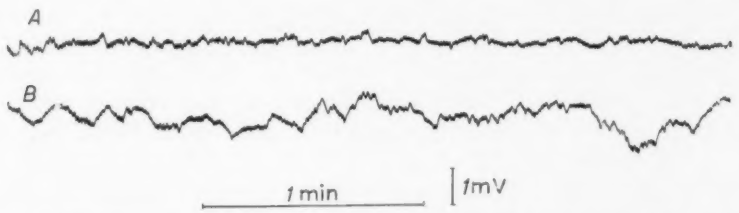

FIG. 5. A dipolar recording of the potential at the top and bottom levels of the cerebral cortex by means of three microelectrodes. *A*: at superficial level to 1·4 mm. *B*: at a depth of 1·4–2·0 mm.

in the rhythm from one and the same area. These may be made to disappear by the application of very careful methods. Consequently, when the recordings are made under chronic conditions with live electrodes, the ultra-slow waves will be most stably recorded when a number of points in the same area are used.

DISCUSSION

It has often been pointed out [7] that the potential arising in the cortex in response to stimulation of receptors has a different sign at the top and bottom layers of the cortex. In work on cats this "inversion" of sign is found at a depth of 0·6–0·9 mm, i.e. at levels III and IV in the cortical layers, where there are accumulations of large neurones with synaptic mechanism transmitting the excitation (Fig. 6).

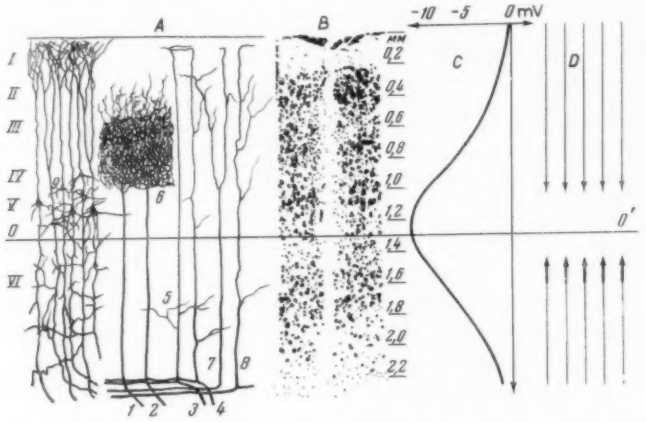

FIG. 6. Comparison of the distribution of the d.c. potential with associated structural features of the cortical layers.

A: a schematic representation of the cortical structure after Lorento de No. 1, 2: specific afferent fibres to the thalamus. 3, 4: non-specific, but numerous afferent fibres. 5: axo-dendrite terminations of non-specific afferent paths, the junctions being unsynapsed. 6: axo-somatic endings of specific afferent fibres. The fine terminated fibres have end-thickenings for contact with the synaptic mechanism. 7, 8: associated fibres. 9: coarse pyramidal mesh of long axons. The respective layers are designated by Roman numerals. *B*: cytoarchetectonic motor zones of the cortex of the rabbit, stained to show nervous tissue. *C*: distribution of the potential in different levels of the cortex. *D*: the directions of the lines of force of electrical polarity in the top and bottom layers of the cortex.

Our data show that the zones of highest quasi-constant potential are localized in greater depths (1·2–1·7 mm, i.e. in levels V–VI of the cortex) and, therefore, do not coincide with the layers showing a concentration of a large pyramidal network. At depths where the levels V–VI occur, it is possible to find variation in the sign of the potential which arises in the cortex in response to stimulus inside the "non-specific" layers of the thalamic nucleus [8]. It is possible that in the layers which have quasi-constant potential at levels V–VI there are significant interacting non-specific afferent pathways in the short axons of the stellate cells [9], in level VI of the cortex.

Non-specific (or multitudinous) afferent fibres (Fig. 6, diagrams 3 and 4), described by Lorento de No [10], contribute collaterals to this level of the cortex, down to

molecular and cytoarchetectonic dimensions. Their endings have an axo-dendritic structure [5]. A number of authors [11] have been unsuccessful in demonstrating anatomical "non-specific" pathways. In distinction from non-specific, the specific afferent nerves have endings of axo-somatic structure (pericorpuscular) forming an intermeshing network of fibres with thickenings, termed "bulbs", which latter have synaptic contact on the body of the cell (Fig. 6, diagram 6). These histological relationships give some basis for the supposition that individual forms of connexion can be created among the non-specific pathways of the cortex. If specific fibres intrinsically transmit impulses through synaptic formations, then it might be possible to assume a different mechanism of transmission for non-specific pathways, the creation of which is indicated by the quasi-constant potential.

The presence of zones of negative potential at definite depths in the cortex makes it possible to assign opposing polarities to the top and bottom regions of the cortex (Fig. 6, *D*). Thus, in the upper layer of the cortex, the electrical vector from the dendritic branchings of levels I and II is directed towards the pyramidal cells in levels IV and V. In the lower levels of the cortex, on the contrary, the pole vector points from the white matter to level V. The potential does not suffer any disturbance but changes evenly in value from level to level.

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The presence of electrical poles together with an unequal distribution of the extracellular electrolytes, makes it possible to show polarizing influences on nervous elements in the different levels. Owing to this, the pyramidal cells in level IV, for example, are found under a greater negative potential than the dendritic mesh of level I.

Analysis of the parameters of the ultra-slow rhythm in different levels of the cortex showed that in both higher and lower levels of the cortex, one can frequently have different amplitude and frequency of the ultra-slow rhythm. In most experiments, in levels I–IV of the cortex, the frequency of the quasi-constant potential was 7–8 c/min, but in levels V–VI the value was 3–5 c/min. The different sign of the potential involving the top and lower parts of the cortex, the different directions of the electrical poles and the divergence of the frequency of the ultra-slow rhythm of quasi-constant potential, all denote intensity of oxidation and glycolysis [9] and have their origin in metabolic processes.

CONCLUSIONS

The direct potential between different points in different levels in the cerebral cortex of the rabbit were found by the insertion of non-polarizing microelectrodes (quasi-constant potential).

According to the depth of insertion in the motor cortex, the potentials registered were all more negative than that of the surface. On further insertion the negative potential was found to diminish (Fig. 1).

The greatest value of the negative potential was found at a depth of 1·2–1·7 mm, approaching the cortical level V, and establishing electrical poles of opposing direction (Fig. 6). During ether anaesthesia the negative zones disappeared.

As previously reported [3] the quasi-constant potential of the cortex has an ultra-slow rhythmic change of period 8–10 sec (secondary rhythm) and 0·5–2 min (minute rhythm). Experiment showed that the ultra-slow rhythm associated with changes in

the quasi-constant potential is found infrequently on the surface of the cortex, but at greater depths within. The frequency and amplitude of the rhythm at different depths were found (Figs. 2, 3).

Significant differences in the parameters of the ultra-slow waves of quasi-constant potential were found, at top and bottom levels of the cortex. On most occasions, the top levels (I, II, III, IV) displayed secondary rhythm of frequency 7–8 c/min and amplitude 0·5–1·5 mV. The lower levels displayed mostly minute rhythm of 3–4 c/min with amplitude 0·8–2·5 mV (Fig. 5).

The sign of the electrical potential originating in the cortex in response to stimulus, contrasts with that of the levels III–IV. The sign of the potential involved, in response to stimulus of non-specific thalamic nuclei, can be changed through the levels V to VI, i.e. at such depth where the greatest value of the negative quasi-constant potential is found. At such depths the parameters of the ultra-slow rhythm becomes changed.

Translated by D. M. ROBERTSON

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SPECTRAL SENSITIVITY CURVES FOR RECEPTORS CONNECTED TO SINGLE OPTIC NERVE FIBRES IN THE FROG*

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(*Received* 21 *November* 1956)

COLORIMETRIC experiments on the frog [1] have shown that its eyes possess two receptors† with different spectral sensitivity curves. In connexion with this, two

† Here, as everywhere else, receptor does not mean a single rod or cone, but a functional system with a particular spectral sensitivity. Various receptors may be separated morphologically, as is apparently the case in the frog (the rods form one receptor, the cones another), but they may occur in the same cell. Thus, for example, each cone apparently contains three receptors in man [3].

* *Biofizika*, **1**, No. 3, 336 [Reprint Order No. BIO 40].

problems arise. Firstly, how does the transfer of information from the two receptors to the brain occur—along the same fibres of the optic nerve, or along separate ones? In other words, does each fibre carry signals from one receptor only, or from both? Secondly, how are we to interpret Granit's results [2]; he believed he was able, using microelectrodes, to detect at least six receptors with different spectral sensitivity curves in the frog.

METHODS

The numbers of receptors connected to single ganglionic cells and their spectral characteristics were determined colorimetrically, i.e. by finding radiations of different spectral composition which were not distinguishable by the given functional element. As a criterion of indistinguishability we used the lack of reaction on exchanging one ray for the other and vice versa.* We assume that each ganglionic cell was connected

A certain radiation of arbitrary spectral composition is chosen as "standard". The general theory of colorimetry implies that any radiation is indistinguishable from some amount of the standard as regards that functional element. If there exists another radiation such that it gives a reaction on being exchanged for the "standard" (or vice versa), then it follows that the given ganglionic cell is connected to at least two receptors of differing spectral sensitivities. By analogy, if the ganglionic cell is connected to two receptors, then any radiation should be replaceable by a certain mixture of two "standard" radiations.

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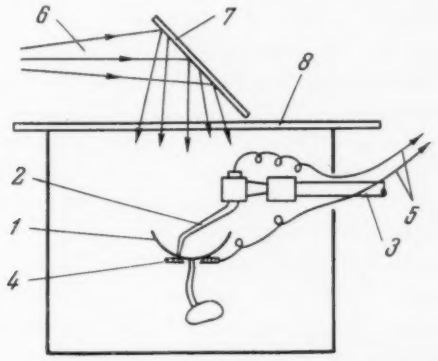

FIG. 1. Layout of experimental set-up. 1—eye preparation; 2—microelectrode; 3—holder for microelectrode, attached to a micromanipulator; 4—platinum electrode; 5—leads to amplifier; 6—light from substitution colorimeter; 7—White matt screen; 8—glass, closing humidified chamber.

The substitution colorimeter which was used in the animal work has been described [1]. In the present work a slight alteration was made: at the exit, in place of an averaging sphere, there was a white matt sheet (Fig. 1).

The work was carried out on isolated eyes from *R. Ridibunda* frogs. The microelectrode was applied to the retina with a micromanipulator. The electrode was of platinum with a diameter of 20–30 μ. The external diameter of the electrode plus glass insulation totalled about 200 μ. Byzov [4] states that such electrodes have the

* In general we shall call radiations interchangeable if rapid replacement of one by another gives no reaction.

best characteristics for detecting potentials from single ganglionic cells of the retina. The second electrode consisted of a platinum wire, applied to the posterior part of the sclera (Fig. 1). The a.c. amplifier had a comparatively narrow pass band (Fig. 2); narrowing the pass band improves the signal-to-noise ratio. At the same time the passage of frequencies up to 500 c/s results in nerve pulses being amplified practically without distortion. A cathode-ray oscillograph was used to observe the pulses, and a magnetophone for recording them. The magnetophone record can later be examined repeatedly with the oscillograph and recorded on photographic paper with a fast mirror oscillograph.

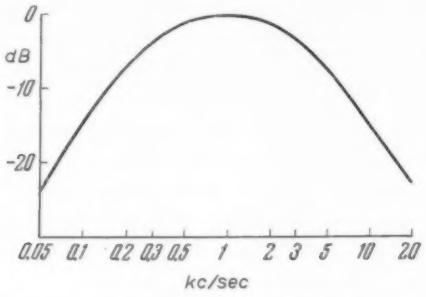

FIG. 2. Frequency characteristic of amplifier.

The introduction of the microelectrode to the nerve elements of the retina was made under electro-physiological control. The electrode was moved about over the retina until the impulses seen on the oscillograph remained more or less of equal height. Sometimes, in place of the impulses coming from the ganglionic cells closest to the electrode, some are seen which come from more distant cells (Fig. 3*a*). But this does not make the results of the experiment any more difficult to interpret because such impulses are easily distinguishable.

The experiments were conducted by illuminating the test field at 10 lux. The eye was adapted to the same brightness because, as preliminary experiments showed, maintenance of this condition in particular facilitates the simultaneous functioning of both of the frog's receptors.

EXPERIMENTAL RESULTS

The first study made was of whether one fibre carries signals from only one receptor, or from both receptors together. Of particular convenience for this purpose were those elements which reacted only on switching on or switching off the light. An example of a record with such an element is shown in Fig. 3. It can be seen that cells which give large pulses emit only when the light is cut off, i.e. they are *off*-elements (according to the terminology introduced by Hartline). It goes without saying that this fibre reacts not only to extinction, but also to any intensity reduction which exceeds the threshold of its contrast sensitivity.

In Fig. 3 is shown a series of records from one and the same *off*-element. Records *a* and *b* show that this element responds to intensity reductions and not to increasing intensities; the *off*-element responded similarly with any spectral composition of the

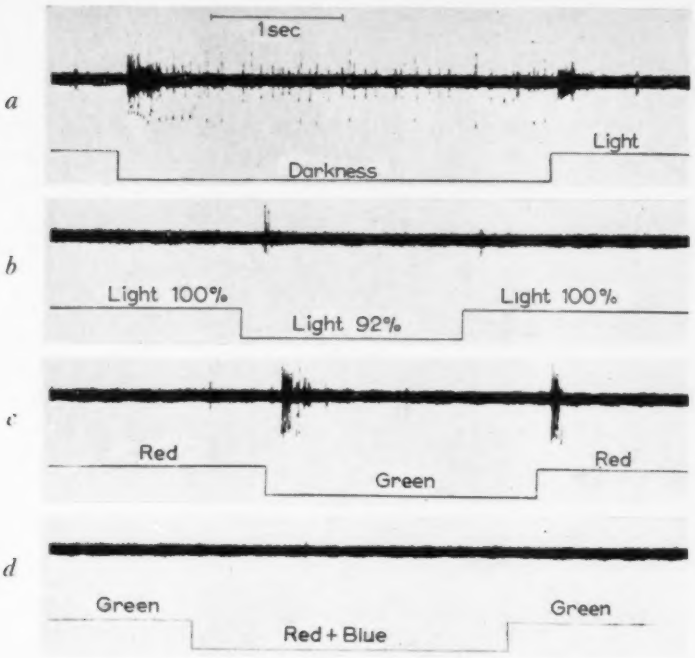

FIG. 3. Series of records with an *off*-element of the retina.

light and to any intensity changes only if the relative spectral composition of the light was not altered.

A different result was obtained by changing the spectral composition of the light; for instance, by replacing a certain green light with a red of different intensity. It appeared to be impossible to select one red of intensity such as to give no reaction on replacing red by green or the reverse. Over a fairly wide range of mean red intensities, impulses were stimulated by both directions of colour change (Fig. 3*c*).* The experiment also showed that there is no blue which is interchangeable with green for this element.

If a given fibre could transmit signals from only one receptor, then we should be able, by an appropriate choice of intensity, to equalize the intensities of any two radiations, including red and green, as seen by that element. The experiment showed that no such equalization is possible. This means that each fibre can transmit information from at least two receptors.

In order to find whether one ganglionic cell is connected to only two receptors or to more, we carried out experiments with two basic radiations—red and blue. The amounts of red and blue in the basic radiations could be selected so that their mixture

* From this, amongst other things, it follows that for the given elements either the concept "brightness" is inapplicable in relation to radiations with different spectral compositions or else that one cannot say it is stimulated only on intensity reduction. Indeed, if we try to define the meanings of "*off*-elements" and "brightness", then, referring to Fig. 3*c*, one would have to recognize that red light is darker than green and at the same time green is darker than red.

is interchangeable with any given radiation, as, for instance, green (Fig. 3*d*). This indicates that the fibre transmits signals from two receptors, because if there were more than two receptors with linearly independent spectral sensitivities then to obtain an interchangeable mixture we should have to use more than two basic radiations

What are the spectral sensitivity curves for these receptors like? Are they identical in different fibres, and are they like the curves obtained by us in previous work on entire frog nerves? To solve these problems we found the colour combination curves

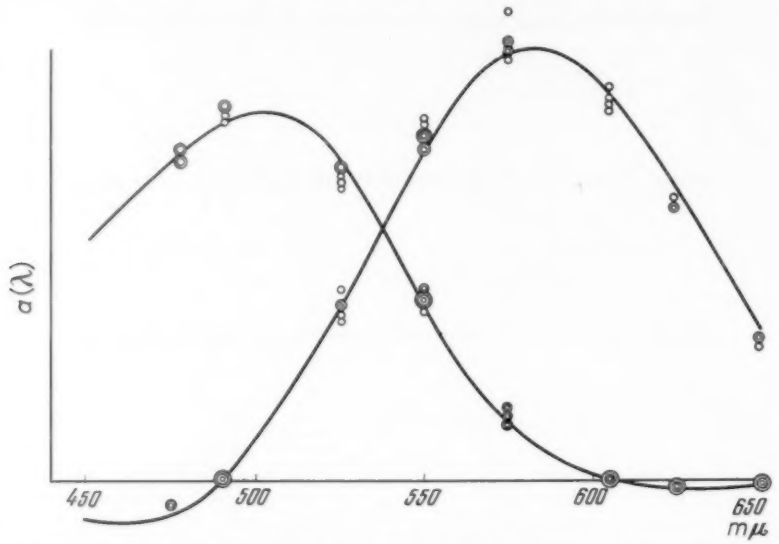

FIG. 4. Colour combination: curves a(λ) for *R. Ridibunda*; lines—for the whole nerve; points—for separate nerve elements.

for single elements. It appeared that within the limits of experimental accuracy various elements were colorimetrically equivalent. The combination curves obtained are shown in Fig. 4. This also shows for comparison the combination curve for the entire nerve. It will be seen that the spectral sensitivities of receptors connected to the separate ganglionic cells do not differ from the spectral sensitivity curves for receptors in the frog's eye previously reported.* In order to confirm for as many fibres as possible whether they give the same matches as the entire nerve, only one point on the curve was determined for each fibre. The points shown in Fig. 4 all therefore relate to separate fibres.

DISCUSSION OF RESULTS

The fact that one fibre of the optic nerve of the frog transmits signals from two receptors is not unexpected. In actual fact histological investigations of the retina provide a basis for thinking that each ganglionic cell is connected to a large group of receptors including cones and rods.

* Strictly speaking the agreement between the combination curves indicates only that some sensitivity curves are linear combinations of the others. However, it is very unlikely that such a correspondence would exist for the whole spectrum if the sensitivity curves did not coincide.

However, there are certain experimental results which could hardly have been predicted. The most important seems to us to be that the signals transmitted by two receptors along one fibre are not interchangeable. The stimulation of one receptor cannot be replaced by that of another.

It was also found that receptors giving out information along one fibre may work simultaneously in a given state of adaptation. The results obtained agree well with the fact that each rod in man contains three daylight vision receptors [3]. Doubts have been expressed about the possibility of transmitting information from several receptors along one fibre. Now that the possibility of such transmission is established experimentally these objections apparently fall away.

In our experiments it was observed that there is a total of two receptors in the frog's retina with different spectral characteristics. Both can be connected to any fibres. However, Granit [2], working with the microelectrode threshold method, believes the frog's retina contains far more modulators. How can this contradiction be explained? We have already stated [5], that by the threshold method one may obtain a spectral sensitivity curve for a receptor only when it is a single functional receptor. If even one other receptor is working simultaneously (and in the frog's retina this is actually the case), the threshold method cannot be used to obtain the spectral sensitivity curves. In this case the threshold method can give all possible combinations of the sensitivity curves for the two receptors as "modulators". Such combinations can vary from one fibre to another for various reasons. For instance, different fibres can be connected to different numbers of rods or cones.

Threshold curves can vary with variations in the state of adaptation (even when the actual receptors have constant spectral sensitivities) and so on. For this reason Granit's observations of a large variety of threshold curves does not indicate a similar variety of types of receptors. At the same time, if the receptors with Granit's "modulator" spectral sensitivity curves in fact existed this would inevitably have emerged from our colorimetric experiments.

CONCLUSIONS

(1) A single fibre of the optic nerve of the frog transmits signals from two receptors of the retina with different spectral sensitivity curves. The signals from these receptors are not interchangeable.

(2) The receptors which transmit signals along a single fibre can work simultaneously under the same conditions of adaptation.

(3) The spectral sensitivity curves for receptors connected to different fibres coincide. They are similar to sensitivity curves for the frog's receptors obtained by transmission of impulses along the entire nerve. There are no receptors with spectral characteristics resembling Granit's "modulators".

Translated by L. BIRKETT

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THE PART PLAYED BY ROD VISION IN THE LIGHT-ADAPTED HUMAN EYE*

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(*Received* 3 *January* 1957)

SPECTRAL colorimetry (the determination of stimulus value curves) is of great importance in the science of colour. Such measurements are the basis of all colorimetric calculations. Investigators usually make it their aim to determine these curves as accurately as possible; if, however, the results of various experimentors are compared, starting with Maxwell, König, Dietrich and so on, right up to Wright and Guild (see, for example, Richter [1]), more or less significant differences can be discovered.

The conditions essential for preserving foveal vision and a constant state of adaptation are usually carefully standardized, especially in more recent work. In spite of this the results often show a scatter which cannot be checked.

Schrödinger [2] long ago suggested a probable reason for these divergences. He stated that colour matches do not remain constant at very low intensities, i.e. in conditions of twilight vision. But the intensity up to which twilight vision continues to have an effect remained uncertain. Schrödinger says: "All the deviations from the laws of daylight vision can be explained satisfactorily by the greater or lesser influence of twilight vision."

It is true that in König's experiments, for example, twilight vision could have played a large part since the work was carried out in a darkened room with a wide field of vision. Similarly, the measurements made by Wright and Guild were carried out in a darkened room and rod vision can be assumed. This is shown most strongly in the "green curve", which is then some mean of the true "green curve" and the curve for twilight vision. Although daylight vision undoubtedly dominates in this case, nevertheless the varying influence of twilight vision causes uncontrollable distortions.

The part played by twilight vision in daylight vision has so far never been accurately measured. In this paper we shall describe experiments of this nature.

Within the fovea centralis, i.e. in that part of the retina which consists only of cones, only three daylight receptors function. Therefore if the field of vision does not exceed 1·5°, it is possible to obtain foveal (cone) stimulus curves in a more or less pure form when adaptation is to light of sufficient intensity (the relevant level of adaptation must, of course, be maintained in the intervals between experiments). Three primary colours—the basic colours of the instrument—are then sufficient to match for any fourth colour.

Outside the fovea centralis there are rods as well as cones: the number of these

* *Biofizika*, **2**, No. 3, 342 [Reprint Order No. BIO 41].

increasing radially. Hence four receptors are working simultaneously in this part of the retina. Therefore it is essential to use four primary colours when determining the "four-dimensional" stimulus curves for these areas.

In the usual colorimetric instruments the compared fields are seen side by side. Colour matching at the retinal periphery is then extremely difficult or even impossible, for the following reasons.

An obligatory requirement in any colorimetric measurement is that definite "colorimetric conditions of comparison" must be produced and maintained during the experiment. It is essential that under the experimental conditions any objectively identical radiations should be visually identical. Conformance with this enables one to judge the uniformity of the retinal state for the compared fields.

The colorimetric conditions of comparison for adjacent fields may be disturbed by variations in adaptation arising during the work, even with normal colour measurements within the foveal rodless area. Therefore while making the measurements one must always take care to restore the colorimetric conditions of comparison (this is usually done by using a standardized grey field in intervals between measurements to equalize the states of adaptation over the retina).

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Conformance with colorimetric conditions of comparison is, strictly speaking, impossible in principle for the peripheral regions of the retina, however. The rod: cone ratio changes continuously from the centre to the periphery, so visual identity of objectively identical radiations is impossible using differently situated regions of the retina. Also when equalizing adjacent fields the line of separation must be fixated, as is done in the usual (foveal) experiments, but extrafoveal experiments are by their very nature incompatible with fixating the dividing line between the fields. This creates considerable difficulty in performing extrafoveal experiments and lowers their accuracies.

Things are different when using our "substitution" instrument [3, 4]. Its principle differs markedly from normal: the object and comparison fields are projected on to the same part of the retina, and may be exchanged at any moment. This "substitution" principle should not be confused with that of the flicker photometer, in spite of some similarities. In the latter an extremely rapid alternation of radiations known to be visually different is employed. The change is made imperceptible by increasing the rate of alternation (critical flicker frequency). In the substitution colorimeter the alternation is on the single-shot principle (two consecutive exchanges can, if necessary, be separated by a considerable time interval), and equality (moment of changeover imperceptible) is achieved by selecting radiations to be compared, i.e. just as in colorimeters using adjacent fields. The colorimetric conditions of comparison are thus always fulfilled automatically since both radiations fall on the same part of the retina.

Using the substitution colorimeter it is easily shown that there are really four receptors operating simultaneously in the extrafoveal retina, and what is more all four operate even at high field brightnesses. (We confirmed this experimentally for a brightness of around 400 apostilb.) If field matching is attempted using peripheral areas, and using three primary colours, it appears that a complete match cannot always be found. But if a fourth colour is used it is possible to obtain very accurate matches in extrafoveal areas. The four-dimensional matches obtained remain

essentially true for the whole retina.* Naturally, there is only one completely defined relationship between the four primary colours in the instrument at which a colour match will be correct, both at the centre and the periphery.

When comparing three-dimensional foveal matches with four-dimensional matches valid for the whole retina, the accuracy is just as in comparing the colour equations of trichromats with those of dichromats, only with a different number of primary colours. The dichromat accepts any three-colour match made by a trichromat, but can select many more combinations from the same three primary colours which are to him indistinguishable from the colour being measured. Only one of these combinations will be a match for both dichromat and trichromat. A very similar situation occurs in the rod-free region of the retina, where three primary colours are sufficient, and in the parafoveal, "four-dimensional", region, where four receptors operate and four primary colours are needed to perform an accurate match.

After it was proved that four receptors operate at the retinal periphery in the light-adapted eye, the nature of the fourth receptor had still to be explained. We could guess, of course, that it must be a twilight receptor, but we wanted to prove this purely colorimetrically.

As one can easily imagine, it is very difficult to select a colour to match a given one using four primary colours, if the match is to be valid both for the centre and the periphery, unless one can make certain assumptions (these assumptions must, of course, be checked). To facilitate the work we therefore used an indirect method.

In a series of preliminary experiments we determined strictly foveally the curves for five subjects using the three completely known but arbitrary primary colours [4]. Then, on the same instrument, we took twilight curves for four subjects. This was done by strictly extrafoveal matches made at low intensities, when the cones do not operate. This curve corresponds basically with the twilight curve $V^1\lambda$ obtained by other investigators, but has a peak at about 520 mμ.

From the three average foveal curves and the twilight curve we calculated the "four-dimensional" combination curves, i.e. the curves that must be obtained for the four primary colours (460, 487, 518, 640 mμ),† if the twilight receptor is added as the fourth.

The amounts of the primary colours computed as giving a match with the test colour were set up on the instrument, so the subjects had only to correct this already nearly exact match by direct comparison. The measurements were made in the following order: after the preliminary calculated settings had been made the observer, viewing a 5° field foveally, assessed the exactness of the set match and made the necessary adjustments.

The subjects could alter the compared fields at will, so fairly accurate matches were obtained. Then the same fields were observed extrafoveally by fixing on a point 10° from the centre of the 5° comparison field. In both cases some adjustments were

* Some deviations due to pigmentation may be found within the yellow spot. The substitution colorimeter differentiates this pigmented region.

† The fact that the primary colours were not strictly monochromatic, because of the need for increased brightness, was taken into account in the calculation. This was possible since the four-dimensional stimulus value curves obtained from the three-dimensional and twilight curves and the four-dimensional final matches were transferred to a set of four strictly monochromatic colours, and compared in this form.

required in the calculated matches first set. By such adjustments a position was found where one combination of the four primary colours matched the test colour over the whole retina. Since the test colours were monochromatic, one or two of the four primary colours entered into the equation with a minus sign, i.e. had to be added to the test colour. Such matches (valid for the whole retina) could always be obtained using four primary colours. Some slight deviations were, however, observed where the centre of the retina was pigmented, as is only natural. Strictly speaking, four-colour matches were correct over the whole retina, except for the yellow spot. Therefore in the appropriate experiments a 2° sector of the 5° comparison field in the yellow spot region was cut off by a screen.

To determine the intensities of the mixed radiations a calibrated photocell was placed at the eyepiece and all five radiations used fell on it in turn. To do this all but one of the colour slits were closed. The intensities of each of the four primary radiations were calculated from the readings of a photocurrent amplifier. Dividing the four primary intensities by that of the test radiation gave figures referring to spectra of equal intensity. Thus we obtained colour matches valid for the whole retina at many wavelengths between 450 and 650 mμ.

In the first series of experiments, seven measurements were made for each point, and in the second and third series from two to five measurements. The average values from the three series were found. These results were then transferred to the monochromatic primary colours (460, 487, 518 and 640 mμ). The four-dimensional curves were constructed for these primary colours, using the three-dimensional and twilight curves (see footnote p. 336). In another series of experiments analogous determinations were made for five wavelengths.

As regards the transfer of the instrumental primary colour readings (the colours

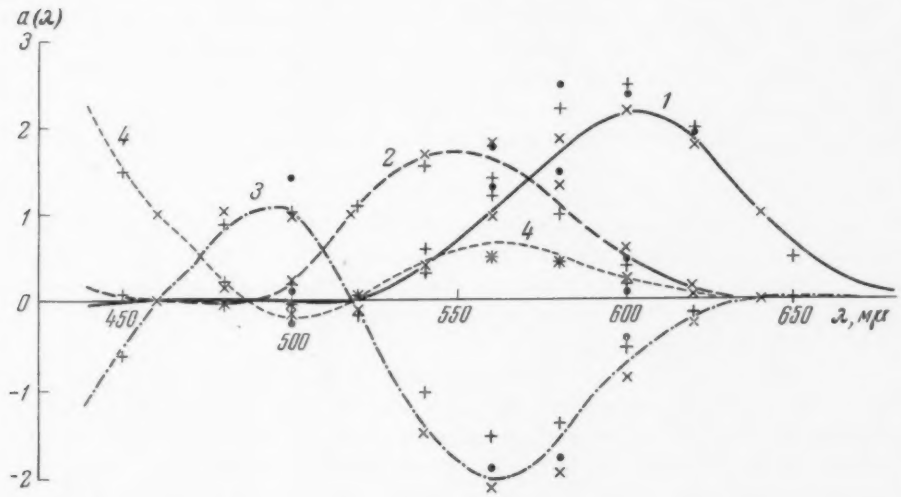

FIG. 1. Colour stimulus value curves for man. Curves calculated from the three-dimensional and twilight vision curves. 1: red—640 mμ. 2: green—518 mμ. 3: blue-green—485 mμ. 4: blue—460 mμ. Curves obtained from four-dimensional matches. Average results. Observer F: X = first series, + = second and third series. Observer S: dots.

being not strictly monochromatic) to strictly monochromatic colours radiations, the following observations must be made: in the second and third series the transfer coefficients were worked out with great accuracy by means of special measurements on appropriate monochromatic radiations using the primary colours in the instrument. In the first series these coefficients were calculated approximately by interpolating results for other parts of the spectrum. The final results obtained are shown in the figure.

The curves constructed from extrafoveal matches correspond reasonably exactly with those constructed from three-dimensional foveal matches and the twilight curve. This means that the fourth receptor occurring in the retinal periphery is, as would be expected, the well-known twilight receptor (rods). As we shall see, it still operates under daylight vision conditions.

The scatter in the direct data is very high, in some cases reaching 20 per cent: the very wide ranges of variation in the four primary colours should be taken into account, since some of the colours are quite similar (blue—460 mμ and blue-green—485 mμ, or the latter and green—518 mμ). Take, for example, the figures for $\lambda = 580$ mμ. In this case a small deficiency of blue-green, mixed with the test colour (i.e. on the "negative side" of the equation), could be to some degree compensated by a deficiency of green + blue on the positive side. The balance cannot be exact, since four-dimensional matches are necessary in the periphery, but the difference would hardly be noticeable. Therefore the scatter in each curve taken individually would be relatively high. This relatively high scatter also affects the accuracies of the coefficients used for transferring the four primary colours to the four monochromatic radiations. The inaccuracies thus cause proportional variations in all calculations using these coefficients. These sources of inaccuracy are difficult to eliminate and must be borne in mind when assessing the results obtained.

Thus the part played by twilight vision in the light-adapted eye has now been established. Twilight vision continues to operate in the peripheral retina during normal daylight vision. With fields greater than 1·5° twilight vision plays a part dependent on the brightness. This can introduce some distortion in normal colorimetry using three primary colours, particularly in constructing stimulus value curves.

CONCLUSIONS

Work with a substitution colorimeter showed that extrafoveal vision is four-dimensional in man. Determination of the stimulus value curves for the extrafoveal receptors showed that the four receptors consist of three foveal (cone) receptors and a fourth rod receptor. The latter operates in the light-adapted eye and when the test field is of considerable brightness.

It was established that four-dimensional colour matches remain valid for the whole retina (with slight deviations within the yellow spot due to the pigmentation). These four-dimensional matches are valid at any adaptation and also for any proportional variations in the intensities of the compared radiations. If matches are found for the retinal periphery using only three primary colours, firstly they will not be satisfactory in most cases and, secondly, will break down very markedly when the adaptation in the proportional intensities of the compared radiations vary.

Translated by J. J. OLIVER

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LARGE BODIES IN CULTURES OF INFLUENZA AND BIRD PLAGUE VIRUSES*

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(*Received* 24 *January* 1957)

IN 1956 we advanced the hypothesis that in the ontogenesis of influenza virus large bodies develop from the classical elementary bodies, these being NV bodies (*niti so vzdutiiami*—filaments with swellings) [1]. Transitional forms are easily demonstrated by electron microscopy.

Other viruses may be expected to show bodies 10 times larger than the elementary bodies, plus transitional forms. We have examined 21 virus strains from bird plague and influenza type D:

(a) True bird plague virus, Rostok, Vienna, Paris, X = 25 and X = 36** strains;

(b) Asiatic bird plague virus, Kuz'minka, Braun, X, Filaret and Possen** strains;

(c) Influenza type D virus Sendai, Akit, Tan, MN, Tok, Si strains (obtained from Japan and England), and strains 390, 914, IZH, 814 and 960 (isolated by Dr. Gerngross in Vladivostok in 1956.†

Standard cultures of the viruses, completely typical by the usual criteria, were studied. All samples were taken from the chorio-allantoic fluid of infected chick embryos. One of the influenza D strains (Akit) was also cultured on a human fibroblast explant in Enders medium. Samples of normal chorio allantoic fluid and of Enders medium served as controls. Samples of influenza virus types A and A[1], obtained from England and isolated in 1956 in Moscow and Stalingrad (eight strains), were used as controls.

A UEM-100 electron microscope was used. The electron microscope specimens were prepared by adsorption on a film [2].

** Strains obtained from the All-Union Institute of Experimental Veterinary Medicine through Doctors A. Ia. Fomina, O. A. Poliakova and A. V. Prokhorov, to whom I express my sincere thanks.

†All material of influenza virus type D was obtained from the Institute of Virology, Academy of Medical Sciences of the U.S.S.R., through Doctors A. S. Gorbunova, V. M. Gnorizova and A. G. Bukrinskaia, to whom I express my sincere thanks. The above workers will give a detailed description of these exceptionally interesting and virologically unique strains in "Voprosy Virusologii", 2, 1957.

* *Biofizika*, **2**, No. 3, 348 [Reprint Order No. BIO 42].

FIG. 1. Asiatic bird plague virus, chorio-allantoic culture. Elementary bodies, intermediate forms and large spherical bodies.

RESULTS

(1) *Asiatic bird plague virus.* Typical elementary bodies were rarely encountered. "Large spherical bodies" were met with in all preparations, and in particularly large numbers in strain Braun (Fig. 1). Filiform and NV bodies were not found.

(2) *True bird plague virus.* Typical elementary bodies (Fig. 2*a*) and normal filaments (~100 μ wide and up to 5–6 μ long) were observed fairly regularly. Occasionally filaments with swellings, very similar in shape and dimensions to the NV bodies in influenza virus (Fig. 2*d*) were encountered. The number found, however, was always too small for the peculiar structures of these filaments to be discerned. In almost all preparations examined "large spherical bodies", which will be described in more detail below, were found.

(3) *Influenza virus type D.* "Large spherical bodies" (Fig. 2*c*) in large numbers, "fine filaments" (Fig. 2*d*) and peculiar combinations of these forms were seen in all preparations without exception. In cultures of the Vladivostok strains NV bodies were also found, but were unusually large, the filaments being more than twice as thick as those of the NV bodies met with in all the other types of influenza virus (Fig. 2*e*). Despite intensive search, forms resembling the usual NV bodies (Fig. 2*c*) and elementary bodies were not found. The large spherical bodies and the fine filaments were exactly the same in chorio-allantoic fluid and fibroblast culture (Fig. 3).

The large spherical bodies were rounded, and very short and had a diameter of 200–1000 μ. On their internal structure they can be divided into three types: (1) homogeneous hemispheres with a relatively high electron opacity, often forming aggregates; (2) a flat, wrinkled, or granular type, sometimes of very low density, as if "hollow"; (3) "cell-like" forms with dense coarsely granular or homogeneous "nuclei" and membranes separated from the "nuclei" by definite gaps. The nuclei vary considerably in size relative to the body. These three types of internal structure are also found in the NV and elementary bodies.

The fine filaments are half the width of the NV bodies but somewhat higher. Their lengths in our preparations were >1·5–2·5 μ, while the NV bodies reached 15–20 μ. The fine filaments appeared either homogeneous (at our resolving power using chromium shadowing) or definitely granular. Frequently chains of granules could be seen, suggesting fragmentation of fine filaments. The fine filaments often formed balls and masses.

The forms which we previously termed "large spherical bodies with fine filaments" attracted particular attention. They have been found frequently in all strains of influenza virus type D, but not so far in the other viruses. Sometimes this combination apparently arises from simple mechanical juxtaposition. It is impossible, however, to ignore instances such as those shown in Fig. 4*a–e*. They give a definite impression that the membrane of the large spherical body has ruptured and that the fine filaments are emerging from it. Sometimes the large spherical bodies are composed entirely of granules similar to those resulting from fragmentation of the fine filaments. It seems highly probable that the large spherical bodies are the forerunners of the fine filaments. These forms are encountered so regularly that serious attention should be given to them.

No bodies of any sort were found in the uninfected control preparations. In the

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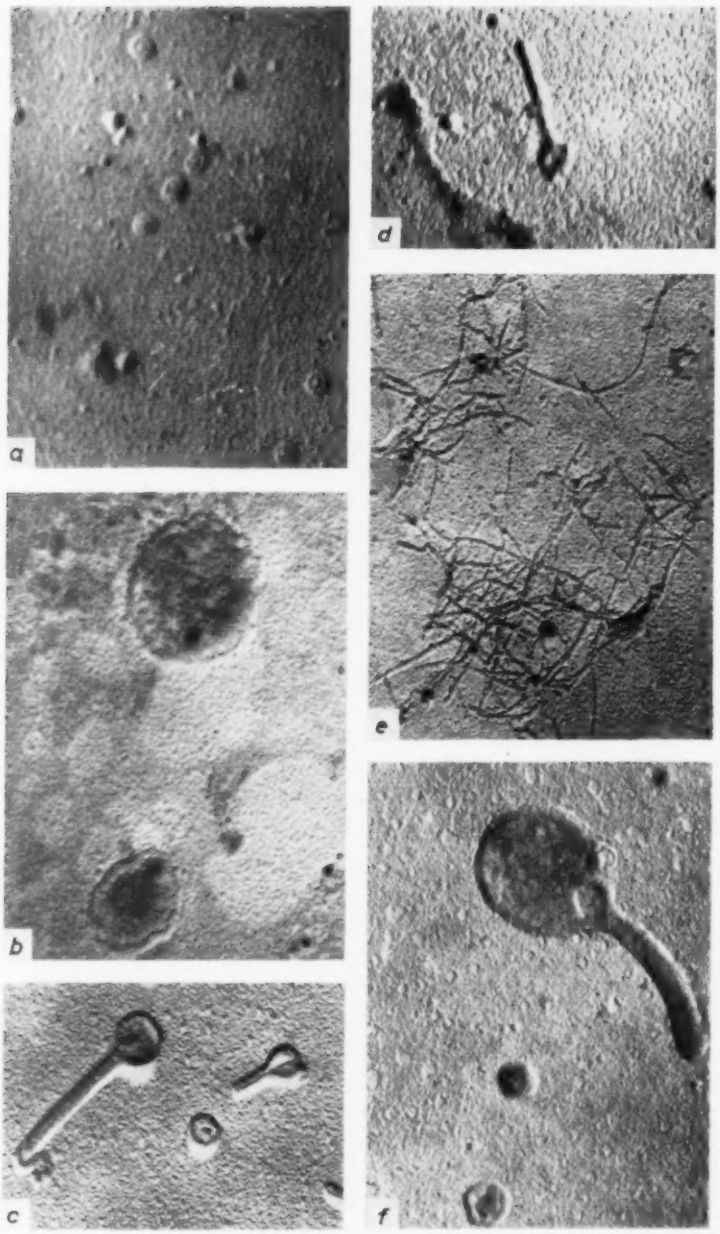

FIG. 2. Photographs: *a*—elementary bodies of true bird plague virus, chorio-allantoic culture; *d*—NV bodies of chorio-allantoic culture of true bird plaque virus; *b*—large spherical bodies, chorio-allantoic culture of influenza virus type D; *e*—accumulations of fine filaments, chorio-allantoic culture of influenza virus type D; *c*—NV bodies from chorio-allantoic culture of influenza virus type A_1; *f*—large NV body and large spherical bodies from chorio-allantoic culture of influenza virus type D.

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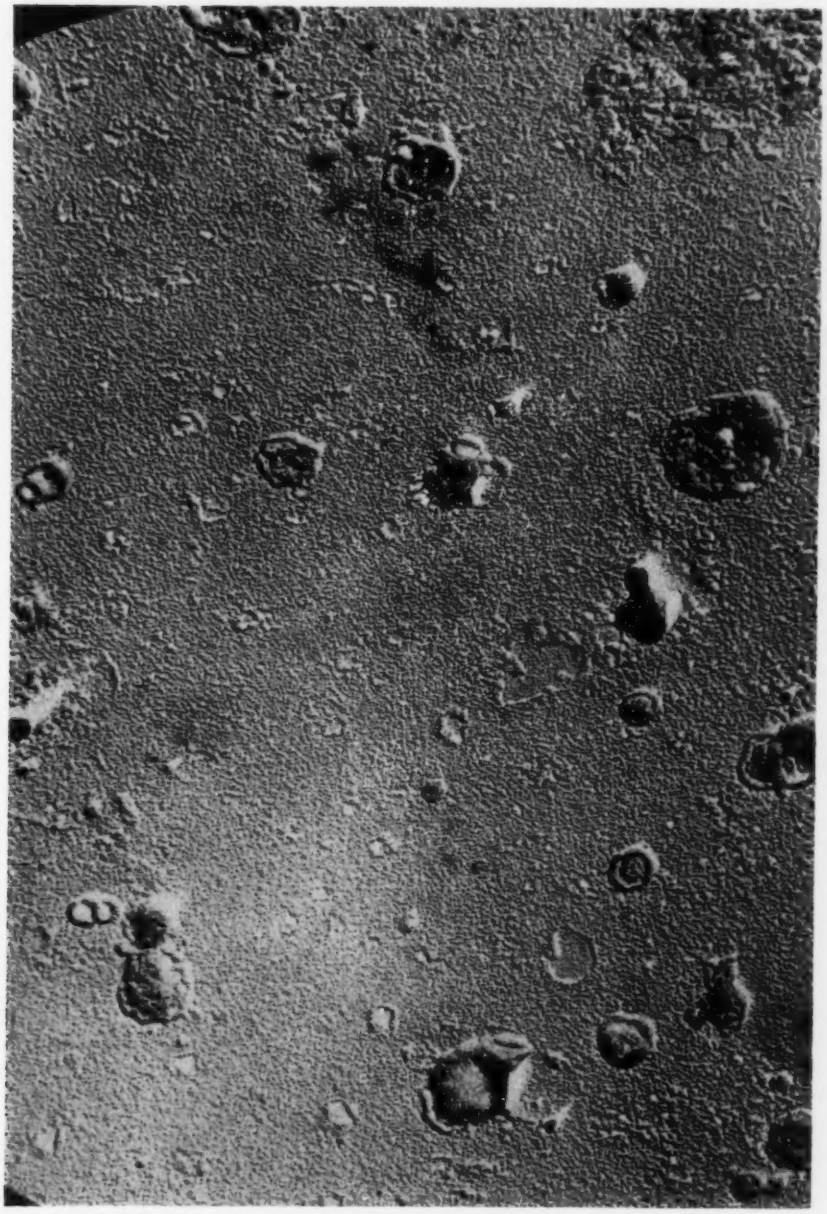

FIG. 3. Influenza virus type D, cultured on human fibroblast explants. Large spherical bodies of various shapes and sizes, variations in their internal structure.

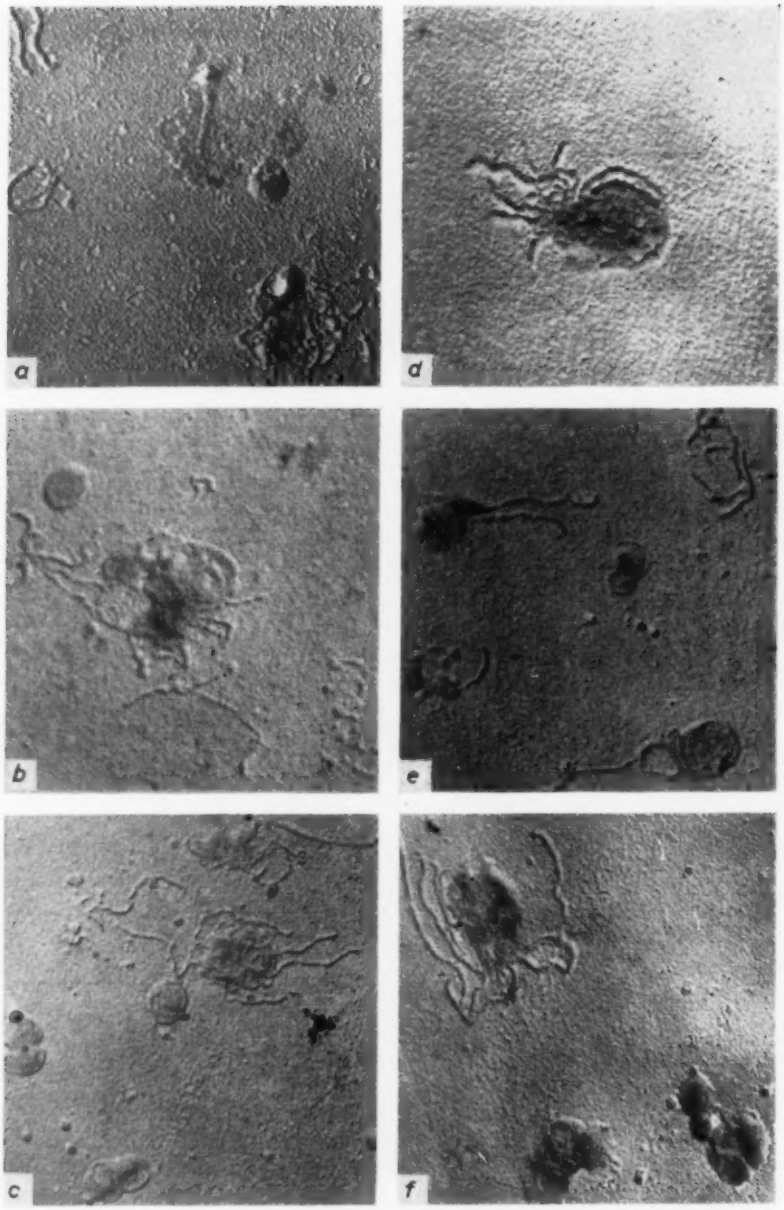

FIG. 4. Large spherical bodies with fine filaments, separate large spherical bodies. Ruptured spheres are seen with homogeneous and granular filaments emerging. Chorioallantoic culture of type D influenza virus. ×21,000.

specimens of types A and A^1 influenza virus strains the pictures seen were the usual ones for these types of virus, and they confirmed our previous observations [1–3].

Thus the 21 strains of bird plague and influenza type D viruses are definitely pleomorphic, coarse bodies being present; the large spherical bodies in bird plague and influenza D virus cultures are practically identical; the fine filaments and coarse NV bodies were only found in type D influenza virus cultures, and the ordinary NV bodies with true bird plague virus.

The points in favour of a virus origin for the coarse spherical bodies and the fine filaments are firstly their regular presence in normal virus cultures and their absence from control cultures; secondly the great similarities in internal structure between the large spherical bodies, the NV bodies and the elementary bodies. Numerous transitional forms between elementary and large spherical bodies of varying shape are seen. A clear morphological connexion between the large spherical bodies and the fine filaments has been demonstrated. Thirdly, the large spherical bodies and the fine filaments from chorio-allantoic fluid and those from the fibroblast culture were morphologically identical. Consequently, they are not the debris of cells destroyed by the virus. Influenza virus does not destroy cells of the chorio-allantoic membrane, and there is not usually any cell debris in the chorio-allantoic fluid. On the other hand, a fibroblast culture is almost completely lysed by infection with influenza virus. We did not, however, find any cell debris in this material and the number of large spherical bodies agreed approximately with the virus titre.

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CONCLUSIONS

The coarse bodies (large spherical bodies, large spherical bodies with fine filaments, and large NV bodies) found in cultures of influenza type D, true and Asiatic bird plague viruses are viral forms. The presence of transitional forms indicates that the coarse bodies originate from the elementary bodies.

Translated by R. CRAWFORD

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THE EFFECT OF ULTRASONIC WAVES ON THE MULTICELLULAR BACTERIUM *CARYOPHANON LATUM**

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(*Received* 23 *October* 1956)

Ultrasonic waves produce morphological, cytological and physico-chemical changes in the microscopic and submicroscopic structures of cells and their separate elements. The latter does not necessarily result in the organism's death. Thus, according to Medvedeva and El'piner [1] some yeast cells (saccharomycetes) retain their ability to grow even on prolonged treatment in spite of definite changes in the physical-colloidal states of the protoplasmic proteins (results of dark-field and fluorescence-microscope studies). Other observations also point to ultrasonic waves affecting molecular complexes in cells. The activities of enzymes bound to cellular structures (mainly the membranes or integuments) are considerably increased by ultrasonic irradiation (invertase, ectoapirase, etc.) [2, 3]. The amount of ergosterol isolable from yeast cells is also increased. Minor functional and biochemical changes apparently govern the lowering of virulence and the rise of immunogenic properties in treated pathogenic micro-organisms—a phenomenon noted by a number of workers [4, 5].

The present communication shows that ultrasonic waves produce functional and cytological changes in microbes which may lead to progeny with different genetically fixed morphological properties.

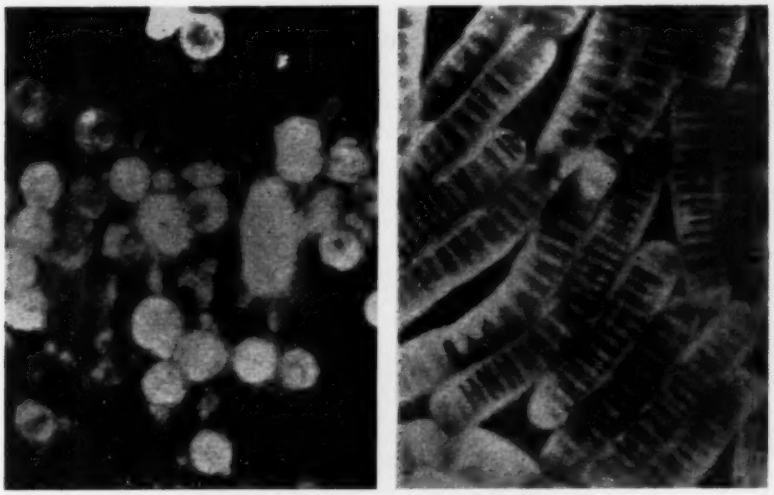
We investigated the multicellular bacterium, *Caryophanon latum*, which proved to be very convenient due to its size and the peculiarity of its protoplast structure [6–8].

A two-milliard suspension of a 24 hr culture in physiological saline was treated with ultrasonics. The test-tube was immersed in an "ultrasonic fountain" in cooled transformer oil containing the ultrasonic source. A concave quartz plate (diameter 6 mm, focal length 100 mm, natural frequency 750 kc/s, intensity 8 W/cm^2, duration of treatment 30 min) acted as the source.

The treated culture was studied by anoptral microscopy; the photomicrographs were with Peshkov's anoptral optics [9, 10]. Dry preparations of the culture, fixed with Bouin's fluid and stained according to Gutstein or combined Giemsa-Gutstein, were also studied.

Fig. 1(b) shows that almost the whole culture was profoundly damaged by irradiation: isolated multicellular individuals were disrupted along the septa into discs; the membrane was damaged. This was confirmed by studies of specially stained preparations. The differences between individual discs were striking: some appeared to be filled with opaque material, others were almost transparent. Both types had a small dark area in the middle—a "navel" or an orifice.

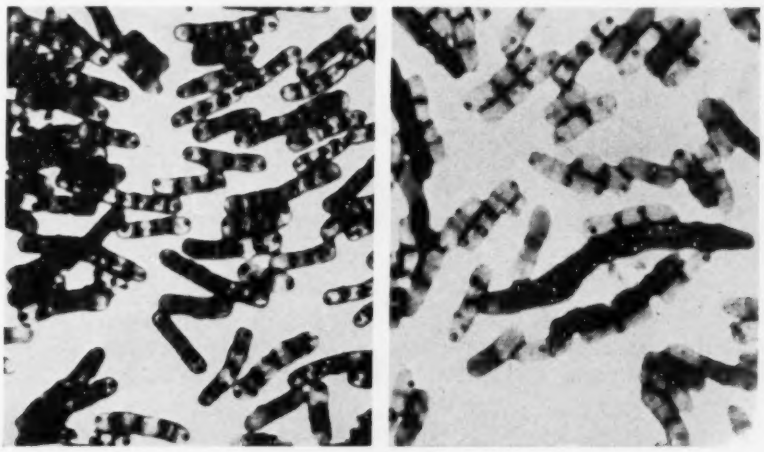
* *Biofizika*, **2**, No. 3, 351 [Reprint Order No. BIO 43].



a b

FIG. 1. (a) *Caryphanon latum*, normal live culture before irradiation. (b) *Caryophanon latum*, live culture directly after irradiation. (Scale shown on Fig. 3.)

Subcultures from treated and untreated cultures on manure agar produced macroscopically identical growths of colonies. Microscopically, however, there were clearly marked cytological differences, observable both in live and in stained preparations. Live control cultures (Fig. 1a) consisted of individuals with a clearly defined membrane and a regular transverse pattern of alternating septa (white) and protoplasts (dark). A stained preparation of the control strain (Fig. 2a) confirms the regular transverse pattern in individuals with deeply stained septa and more delicately stained membrane. Granules—two in each cell—are found on both sides of each septum. Whether these structures are related to chondriosomes is difficult to say.



a b

FIG. 2. (a) Dry preparation of *Caryophanon latum*, obtained by Gutstein's method, normal culture. (b) The same, progeny of an irradiated culture. (Scale on Fig. 3.)

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The presence of a central axial line, more dense than protoplasm, was also to be noted; this was seen in shrunken, fixed individuals (Fig. 2a and b).

The progeny of treated cultures differed sharply from those of the control; most individuals of the former showed indefinite borders, as though the membrane was absent. Many bacteria have large, optically empty areas in the middle and at the ends. The structures of the cells appeared in greater detail and more clearly (Fig. 3).

The basic differences between the progeny from treated and untreated cultures were the much smaller number of septa in each individual in the former and the extremely thin membrane. This latter was apparently elastic, since granules seemed to protrude from the body of the organism, an occurrence not found in the progeny of the control (Fig. 2b).

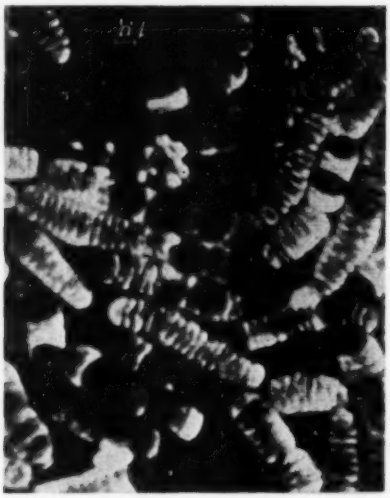

FIG. 3. *Caryophanon latum*, progeny of an irradiated culture, live preparation.

In treated strains the granules vary from one to three and do not occur on each side of the septa, as in normals. The question naturally arises as to what causes the morphological and cytological changes in the progeny of treated cultures. It is known [11] that ultrasonic waves, by "loosening" intracellular complexes, impair metabolic processes. Such impairment may apparently result in progeny of different functional and morphological properties. Further experiments are being carried out along these lines.

CONCLUSIONS

(1) The multicellular bacterium *Caryophanon latum* was disrupted into individual cells or discs after 30 min of ultrasonic irradiation.

(2) The treated culture, subcultured on manure agar, produced a growth macroscopically identical with that from a normal one.

(3) The progeny of a treated culture were morphologically and cytologically different from those of an untreated one.

(4) The changes were observed in five generations; it may therefore be assumed that they are hereditary.

The authors are deeply grateful to Prof. M. A. Peshkov for his valuable guidance during the present investigation.

Translated by I. GORE

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VARIABILITY OF *BACILLUS PERTUSSIS* CAUSED BY ULTRASONIC WAVES*

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(*Received* 23 *October* 1956)

WE have previously shown [1–3] that *B. pertussis* is relatively easily disintegrated by exposure to ultrasonic radiation. The cell contents enter the surrounding medium and a highly toxic preparation is obtained containing the microbe's endotoxin. Moreover, the culture itself undergoes certain changes, losing its virulence whilst retaining its immunizing capacity. This result is obtained even when only an insignificant fraction of the micro-organisms are disintegrated, the other cells being unchanged microscopically and growing on subsequent culturing. A certain polymorphism in their staining was noted, however. Data adduced in the present paper show that, in certain conditions, irradiated *B. pertussis* acquires altered morphological and functional characteristics which are inherited.

In our work we used a dry-stored *B. pertussis* culture. From time to time it was cultured on to artificial media and the culture tested for virulence and toxicity.

A 5 ml suspension of the microbe culture (standardized by intestine test) in physiological saline was irradiated under the following conditions: frequency 700 kc/s, intensity 8 W/cm² of radiating surface. The ultrasonic radiator was a quartz plate. Exposure times were 30, 60 and 120 min.

A 30 min exposure induced little change visible under the microscope in *B. pertussis*; preparation exposed for 120 min turned into a uniform detritus which reproduced no

* *Biofizika*, **2**, No. 3, 354 [Reprint Order No. BIO 44].

further. A 60 min exposure produces a massive cell break-up. Cultures from the latter suspension gave a special type of growth, as single colonies. Abundant growth was found after 30 min irradiation.

As a rule the surviving micro-organisms showed normal specific growth if inoculated into a Bordet–Jangou medium immediately after exposure. The culture thus grown subsequently reproduced again in a normal way following repeated exposure. Even multiple exposure immediately followed by culturing produced cultures with morphological, toxic and immunogenic properties identical with those of the original. With the original (intact) culture a intradermal injection of 1 ml or 5×10^8 irradiated *B. pertussis* cells into rabbits, produced extremely sharp local necrosis (necrozing action). The lethal action of an irradiated culture showed itself in a similar way: as with the original culture, mice injected intraperitoneally with 1 ml or 5×10^8 irradiated bacteria nearly all died (lethal action of *B. pertussis*).

A slightly different technique produced completely different results. After irradiation the suspension in physiological saline (5×10^9 per ml) was left to stand for 24–48 hr in a refrigerator at +4°C. In other words, cells which had survived 30 min irradiation intact were left in direct contact with cell fragments. At the end of the period, *B. pertussis* was cultured on Bordet–Jangou medium and a 2 day culture thus obtained was again irradiated for 30 min. This culture was similarly stored for 24–48 hr at 4°C, then cultured and once more irradiated, the entire sequence being repeated from five to six times.

The resulting culture had acquired a number of new characteristics. Morphological changes were manifest. The bacillus became thicker, appearing swollen; in many cases globular protrusions appeared at one or both poles. Quite frequently the cells appeared optically empty (see Fig. 1). Culture properties also underwent a change: a dryish growth was noted (on Bordet–Jangou medium), giving large white colonies. Suspensions in physiological saline quickly settled to the bottom of the test tube leaving the solution transparent (R-form).

Study of toxicity and virulence produced especially interesting results. Table 1 shows that the necrotic activity of the *B. pertussis* culture was significantly lower than

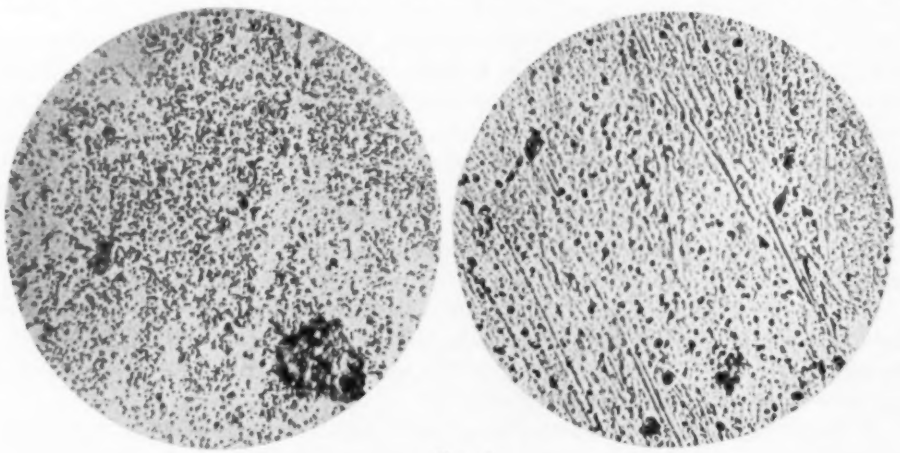

FIG. 1.

that of the native control culture (this was tested by intradermal injection in rabbits). The culture's virulence had diminished particularly noticeably.

TABLE 1. TOXIC AND VIRULENT PROPERTIES OF AN ALTERED CULTURE OF *B. pertussis*

Bacterial culture	Diameter (in cm) of skin necrosis after injecting rabbits with bacteria		Lethal action of culture after injecting mice with bacteria*		
			Intraperitoneal		Into nose membrane
	2×10^8	10 ml	2 ml	1 ml	5×10^7
Grown after 5- to 6-fold exposure to ultrasonic waves, followed by culturing	Necrosis 1 Necrosis 1 Necrosis 1 Necrosis 1	No necrosis As above Necrosis 0·5 As above	33/40	38/40	21/55
Native control	Necrosis 2·5 As above Necrosis 2 As above Haemorrhage 2	Necrosis 1·5 As above As above As above Haemorrhage 1·5	0/30	13/62	8/100

* Numerator gives number of mice which survived; denominator gives number of mice injected.

Most of the mice (71 out of 80) given 1–2 ml intraperitoneal injections of culture cells survived. Identical injections of control culture resulted in the survival of only 13 out of 92 mice.

Optimal dose conditions were applied to test the immunizing potential of exposed *B. pertussis*. The minimum immunizing dose was determined by a fivefold preparation of the mice. Mice were immunized by intraperitoneal injections at 4–5 day intervals. The immunizing potential was judged from Zdrodovski's intraperitoneal test.

TABLE 2. IMMUNOGENIC PROPERTIES OF AN ALTERED CULTURE OF *B. pertussis*. TOTAL DOSE: *a*—10×10^7 BACTERIA; *b*—5×10^7 BACTERIA

Bacterial culture	Number of immune mice after fractional immunization*†	
	a	*b*
Obtained from 5- to 6-fold exposure to ultrasonic waves, followed by culturing	19/25	17/23
		12/13
Native control	15/16	3/19*

* Mice not previously immunized.

† Numerator gives number of immune mice; denominator gives number of mice injected.

If a quantity of preparation sufficient to immunize 75 per cent of the injected mice is taken to represent the minimum immunizing dose, then about 1×10^8 exposed *B. pertussis* cells forms such a dose. And in fact, as shown in Table 2, the total dose of exposed culture required to produce significant immunity on triple immunization contains $5–10 \times 10^7$ cells.

Out of 48 mice injected with three or four times the lethal dose of the native control culture, 36 proved immune. A native intact culture produced immunity in 27 out of 29 mice. However, an irradiated culture, unlike a native one, is not toxic and is therefore more suitable as an immunizing agent.

A *B. pertussis* culture with similar properties was also obtained after direct repeated (five to six times) culturing of an irradiated specimen on Bordet–Jangou medium containing cell break-up products. In this case, however, it was necessary to irradiate before each sowing. Culturing alone did not produce the desired effect.

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CONCLUSIONS

(1) Exposure of *B. pertussis* to ultrasonic waves produced a culture with altered morphological, toxic and immunogenic properties.

(2) This altered culture was obtained by keeping intact micro-organisms and cell fragments in contact. A markedly altered culture of *B. pertussis* was obtained by irradiation, storing in a refrigerator at 4°C, and culturing.

Translated by R. ZATORSKI

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REFLECTING PROPERTIES OF BUTTERFLY WINGS AND ROLE OF ULTRA-VIOLET RAYS IN THE VISION OF INSECTS*

G. A. MAZOKHIN-PORSHNIAKOV

(*Received* 1 *September* 1956)

INTRODUCTION

THE colours of the integuments of insects, which are often very striking in diversity and saturation, have long been a matter of interest to investigators. The subject has been studied from two main aspects: the origin and nature of the colouring, and its biological significance in the life and behaviour of insects. By exact chemical and physical methods, including the electron microscope, the dual nature of the colouring has been established from the first point of view: as pigmentary and as physical or structural, related to interference and total internal reflection in the chitin structures [1–6].

* *Biofizika*, **2**, No. 3, 358 [Reprint Order No. BIO 45].

The second aspect (the more difficult) depends on a whole series of ecological and biophysical data, not all of which are as yet known to science. We still lack the accurate data needed to evaluate the biological value of coloration as a means of recognition and disguise for its owners; firstly, on the colour vision of insects and, secondly, on the reflecting properties of their integuments and of the objects which form their natural environment. We still do not know the spectral sensitivity curves for insect eyes and have thus no means of judging which spectral components, in what proportions, are differentiable to insects. All we have to rely upon so far are the curves for the colours perceived by a few species of insects. These curves have their maxima generally in the long-wave ultra-violet, which seems to indicate high sensitivity to ultra-violet radiation and, therefore, ability to see objects which reflect these rays. In the case of bees (Kühn [7], Lutz [8]), bumble-bees (Kugler [9]) and some butterflies (Crane [10]), this property has been demonstrated by training the insects to distinguish colours.

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Bearing in mind that insects can perceive the long-wave ultra-violet, it is of special interest to study the colouring of objects which appear indistinguishable to human eyes but which have different coefficients of reflection in the ultra-violet.

These objects can be visually distinguished by insects and we must bear the fact in mind in evaluating their behaviour and the part played by the colorations of insects and plants in nature.

Some work has already been done in this and similar directions. Lutz [11] published photographs of certain types of insects taken in ultra-violet light. Brues [12] photographed a number of American butterflies through a blue filter which was partially transparent to ultra-violet light but absorbed orange and red. In the opinion of the author these photographs convey some idea of how the patterns of butterfly wings may differ in brightness to insect eyes. Makino *et al.* [13] studied the reasons for the different ultra-violet reflection coefficients of the wings of male and female cabbage butterflies (*Pieris rapae* L.). After extensive investigation of the visible and ultra-violet reflection coefficients for the wings of tropical butterflies, Crane [10] concluded that these rays play an insignificant part in colours perceived by insect eyes. Lastly, in 1954, we [14] published a relation between ultra-violet reflection parameters of butterfly wings and their sexual and visual characteristics, and also on the relation between the ultra-violet reflection coefficients of bumble-bees and their geographical distributions.

The present work was undertaken to determine the ultra-violet reflection coefficients for European and tropical butterfly wings and to elucidate the effect of these rays on wing coloration. The differentiation of colour patterns by insects was studied via comparisons against a background, a method neglected by other workers.

PROCEDURE

Photographic methods were used for the photometry of butterfly wing patterns. The negatives are compared with a standard wedge of known reflection coefficients taken on the same frame (Fig. 1). Ultra-violet photography as a method of studying the reflecting properties of objects has the great advantage of permitting fields with different reflection coefficients to be differentiated rapidly, which is hardly feasible with a spectrophotometer.

A "Zorkii" camera with an "Industar 22" lens was used with a positive (non-sensitized) cine-film. A PRK-4 quartz-mercury vapour lamp illuminated the test objects and standard wedge. A Schott UG=1 filter, transparent to the near ultra-violet (maximum transmission around 360 mμ) but opaque to the visible region apart from the far red, was mounted before the lens during recording. This equipment gave photographs in monochromatic light at 365 mμ (a strong line in the ultra-violet spectrum of mercury).

The standard wedge was made of chalk and chalk–soot mixtures and was photometered at 365 mμ with a Pulfrich spectrophotometer adapted for use with a cadmium vacuum photocell. The scale had seven steps corresponding to reflection coefficients

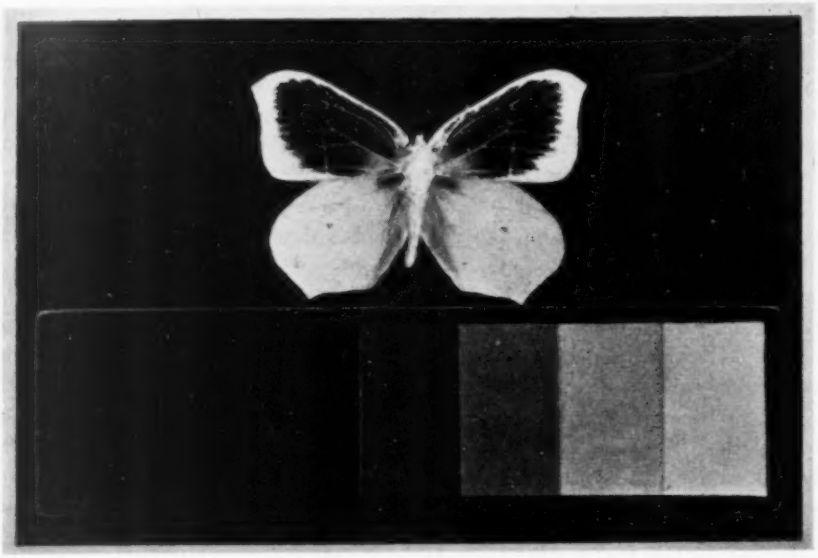

FIG. 1. Negative of *Gonepteryx rhamni* L. and Standard Wedge.

of 70, 60, 40, 22, 11, 6 and 3·5 per cent. Intermediate values were determined by interpolation.

The densities of the image and of the wedge taken on the same frame were read on a photoelectric microdensitometer. The field stop of the microdensitometer can be used to estimate densities on both large and small areas of a wing. The use of small areas was facilitated by the relatively large (1 : 3) linear magnification of the negatives.

The relative accuracy of this photographic method was about 6 per cent for high reflection coefficients and 10 per cent for low.

With butterflies having very complicated patterns of small dots or lines the reflection coefficients were determined for the pattern as a whole, without delimiting its separate components. For areas of strongly variable colour, e.g. the rear of the hind wing in the Cabbage White butterfly (*Pieris*), Table 1 gives only one coefficient relating to one of the specimens studied.*

*As far as possible, specimens from the Zoological Museum, Moscow University, were used, for which the author's thanks are due to the Curator of the Entomological Section, A. N. Zhelokhovtsev.

DISCUSSION OF RESULTS

The ultra-violet reflection coefficients for the upper and lower wing surfaces of over 50 male and female butterfly and moth species belonging to 12 genera were measured. The wing areas and their coefficients of reflection (for 365 mμ) are given in Table 1. Wing areas with similar hatchings have equal reflection coefficients.

Generally speaking, the upper and lower wing surfaces reflect ultra-violet light only weakly, but some butterfly and moth species have upper wing surface reflection coefficients of 40–75 per cent, e.g. the bright yellow or orange patterns of *Gonepteryx*, *Catopsilia*, *Dercas*, blue *Lycaena* and perfectly white *Nygmia*, *Spilosoma*, male *Hepialus numuli* L., etc. The lower wing surfaces almost always have lower reflection coefficients than the upper.

More frequently some pattern components have higher reflection coefficients, e.g. the white portions near the wing base of female *Aporia crataegi* L., white dots on *Apatura ilia* Schiff and *Limenitis populi* L., greenish-yellow spots on the lower wing surfaces of *Pieris brassicae* L. and *Hypermnestra helios* Nick, etc.

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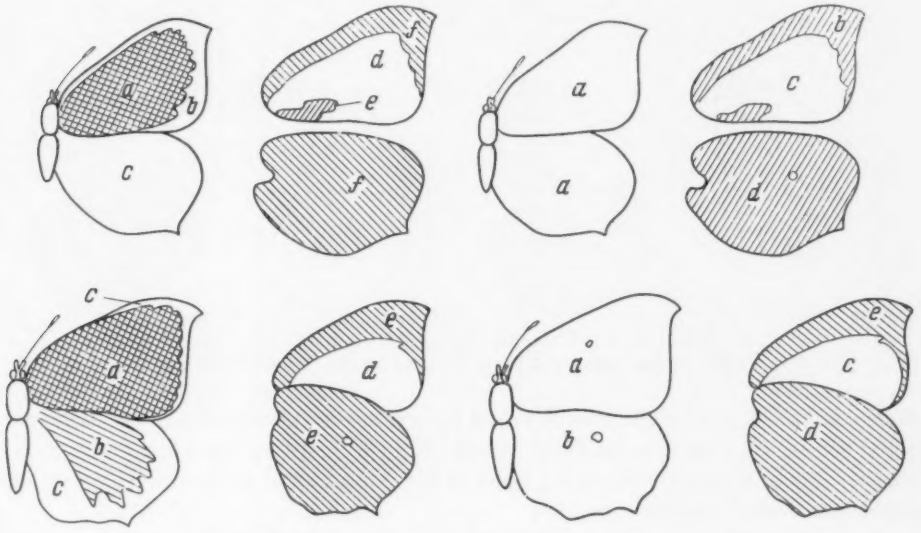

FIG. 2. *Gonepteryx rhamni* L. (upper row) and *G. cleopatra* L. (lower row).
Left—males. Right—females.

In many European and tropical forms of *Pieris*, *Argus* and some *Nymphalidae*, *H. numuli* L., the ultra-violet reflection coefficients differ considerably between males and females [14]. The sexual dichroism invisible to the human eye becomes clearly evident in *Gonepteryx* (Fig. 2) and *Aporia* (Fig. 3). For instance, the male *G. rhamni* L. with its monotone lemon-yellow coloration has an upper-surface fore-wing coefficient of about 40 per cent, while in the greenish-white female both wings reflect only 7·5 per cent. The perfectly white male and female *A. crataegi* L. differ in that a part of the fore-wing in the female reflects 15 per cent while both wings of the male reflect only 3 per cent. Smaller differences, of the order of 3·5 per cent, are observed between male and female *Pieris*, while in blue *Lycaena* they amount to 65 per cent.

The diagrams show that wing patterns seen in ultra-violet light often do not correspond (e.g. in male *G. cleopatra* L. (Fig. 2) and *C. philea* (Fig. 4, *A*)) with those in visible light or may even be entirely invisible (e.g. in male *C. rhamni* L. and *L. icarus* Rott. or female *A. crataegi* L.). The reason is that butterfly wing parts similar in visible light (for man) may differ appreciably in their ultra-violet reflection or, inversely, wing parts different to the human eye may have equal coefficients of reflection in the ultra-violet.

The fact that wing patterns invisible to the human eye are perfectly visible to the

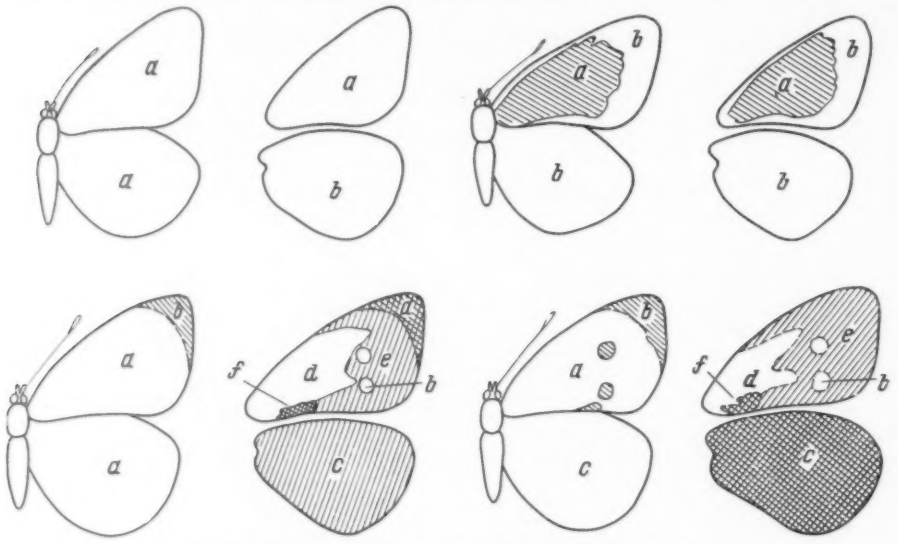

FIG. 3. *Aporia crataegi* L. (upper row) and *Pieris brassicae* L. (lower row). Left—males (upper and lower wing surfaces). Right—females.

insects is an instance of the special visual perceptions of insects caused by their eyes being sensitive to ultra-violet light. Such "hidden" patterns are apparently rare in insects. At least Crane [10] found none in his photometric studies of 41 species of butterflies from Trinidad.

It would seem that yellow, orange or blue wing areas reflect ultra-violet light most strongly. This coloration appears to be both of pigmentary and structural origin. White surfaces (reflecting the entire visible spectrum) may absorb much of the ultra-violet (*Pieris*, *Aporia*) or hardly any (*Spilosoma*, *Arctornis*, *Nygmia*). Black areas, absorbing the visible spectrum, also absorb ultra-violet rays (*Argus*, *Skipper*). The central spots on the fore-wings of *C. menippe* Hbh. and some other species are exceptions.

White coloration with strong ultra-violet absorption is found only in butterflies (*Rhopalocera*) while nocturnal moths (*Sphinx*, Tiger Moth and Spinner Moth) have high reflection coefficients, ~25–55 per cent. Since the light of the night sky (about 75 per cent of all light reaching the earth when there is no moon) contains a high proportion of ultra-violet [15], the strong ultra-violet reflections may help moths to find each other by vision.

The green wings of certain forms (*Gonepteryx*, particularly *Catopsilia*) fluoresce strongly under ultra-violet illumination. The fluorescence colours of areas absorbing in the ultra-violet are the same as the real ones. Hence the wings appear very bright and must present a sharp colour contrast between absorbing (fluorescent) and reflecting areas to the insects.

Androconia patches—odoriferous structures peculiar to some male lepidoptera—in *Pieris* and some *Argus* reflect ultra-violet light more strongly than the surrounding wing parts. In the other species, e.g. *Satyrus circe* F. (Fig. 4, *E*) or *C. philea* (Fig. 4, *A*),

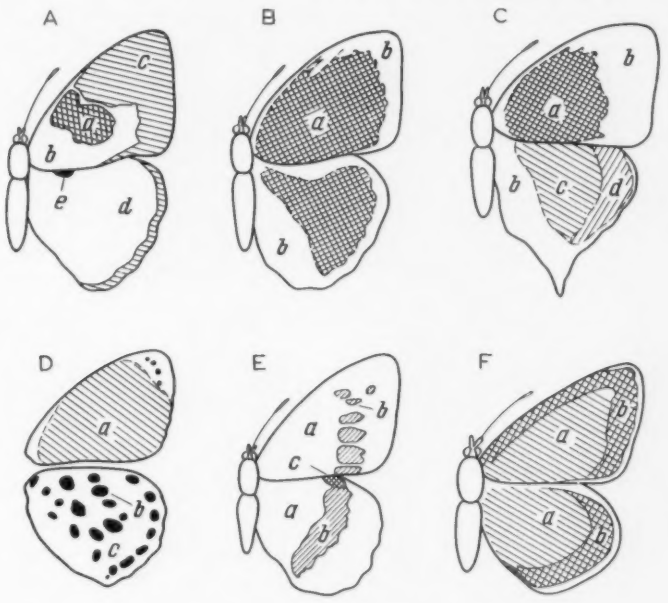

FIG. 4.

A—*Catopsilia philia* ♂;
B–*C .argante* F.;
C—*Dercas verhuellii* Hav.;
D—*Argynnis aglaja* L.;
E—*Satyrus circe* F. ♂;
F—*Lycaena icarus* Rott ♂.

the patches are hardly distinguishable by eye from the rest of the wing but stand out sharply on ultra-violet photographs. This photographic method of rendering androconia patches visible should be useful to morphologists and taxonomists.

The resemblance of the protective (masking) coloration of the undersurface of the hind-wing and top of the fore-wing, characteristic of *Pieris* (Fig. 3), *Argynnis* (Fig. 4, *I*) and other species, is preserved in ultra-violet light, as indicated by their having the same reflection coefficient for 365 mμ.

Most of the distinctively coloured nocturnal hawk, noctuid and geometer moths have practically the same appearance in ultra-violet as in white light, i.e. the relative brilliances of the wing patterns are preserved also in ultra-violet light.

Lepidoptera have colour vision, particularly *Papilio*, *Pieris* and Nymphalidae [10, 16–18]. According to Knoll [19] even nocturnal lepidoptera, particularly hawk moths, can distinguish colours, and their eyes, like those of most insects, are sensitive to ultra-violet light.*

* Wigglesworth's [20] statement that there are no data on the sensitivity of butterflies to ultra-violet rays must now be considered out of date since the publication of Crane's [10] and Mazokhin-Porshniakov's [21] work.

TABLE 1. COEFFICIENTS OF REFLECTION FOR

Butterfly	Upper wing surface									
	Coeff. reflection, per cent					Colour (for human eye)				
	a	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*d*	*e*
Aporia crataegi L. ♂ (Fig. 3)	3					White				
Aporia crataegi L. ♀ (Fig. 3)	15	6				White				
Pieris brassicae L. ♂ (Fig. 3)	>1	2				White	Dark grey			
Pieris brassicae L. ♀ (Fig. 3)	4·5	2	3·5			White	Dark grey	Greenish-white		
Gonepteryx rhamni L. ♂ (Fig. 2)	40	3·5	3·5			Lemon-yellow				
Gonepteryx rhamni L. ♀ (Fig. 2)	7·5					Greenish-white				
G. cleopatra L. ♂ (Fig. 2)	45	27	4			Orange	Lemon-yellow			
G. cleopatra L. ♀ (Fig. 2)	5	3·5				Greenish-white				
Catopsilia philea ♂ (Fig. 4, A)	74	2	3	2	11	Reddish-orange	Lemon-yellow			Pale yellow
C. argante Fab. (Fig. 4, B)	62	6				Orange				
Dercas verhuellii Hav. (Fig. 4, C)	70	4	2	8		Lemon-yellow				
Hypermnestra helios Nick ♂ (Fig. 5, *d*, *e*)	7·5	3	10			Pale yellow	Black or grey	Red		
Limenitis populi L. ♂ (Fig. 5, A, B)		≈1			35		Dark brown			White
Apatura ilia Schiff ♂ (Fig. 5, C, D)	11	3	40	25		Dark brown		White		
Argynnis aglaja L. ♂ (Fig. 4, D)										
Satyrus circe F. ♂ (Fig. 4, E)	≈1	3	8			Dark brown	White			
Lycaena icarus Rott. ♂ (Fig. 4, F)	60	70				Sky-blue				
Lycaena icarus Rott. ♀	Over entire field 3·5					Dark brown				
Cupido virgaureae L. ♂	Over entire field ~1					Fiery-red				
Cupido virgaureae L. ♀	Over entire field 3·5					Orange and black				
Hepialus humuli L. ♂	Over entire field 55					White				
Arctia hebe L. ♂ (Fig. 5, *g*)	3	20	6			Velvety black	White	Cinnamon red		
A. caja L. ♂ (Fig. 5, H)	3	15	4·5	10		Brown	White	Reddish-orange	Dark grey	
Spilosoma urticae Esp. ♂	About 40					White				
Spilosoma urticae Esp. ♀	About 37					White				
Porthetria dispar L. ♀	About 21					Brownish-white				
Actornis l-nigrum Muell. ♂	About 37					White				
Stilpnotia salicis L. ♂	About 25					White				
S. salicis L. ♀	About 25					White				
Nygmia phaeorrhoea L. ♂	About 40					White				
Nygmia phaeorrhoea L. ♀	About 37					White				

λ = 365 mμ FOR BUTTERLY WINGS

Lower wing surface

Coeff. reflection, per cent						Colour (for human eye)					
a	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*d*	*e*	*f*
7	6					White					
15	7					White					
3·5	1	6	1	3	21	Greenish-yellow	Black	Greenish-yellow	White	White	White
	3	12	3	8	21	Greenish-yellow	Black	Greenish-yellow	White	White	White
			6	21	9				Lemon-yellow	Greenish-white	
	21	5	21				Greenish-white	White	Greenish-white		
			8	20					Yellow	Greenish-yellow	
		8	23	20				White	Greenish-white		
Over entire field 2–3						Orange					
Over entire field 2–3						Orange-yellow					
Over entire field 2–3						Lemon-yellow					
13	6	30	3·5			Pale yellow	White	Greenish-yellow	Black		
30	≈1	7				White	Black and grey	Green			
	3	21	11	40	≈1		Cloudy-orange	Light grey	Cloudy violet	White	Black or greenish
6	40	2				Reddish-brown	Cloudy-orange Silvery-white	Yellow or greenish			
Over entire field 10–12						Bluish-grey					
,, ,, ,, ,,						Velvety-grey					
Over entire field 3·5						Cloudy-orange					
,, ,, ,, ,,						,,	,,				
Over entire field 10						Grey					

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Lepidoptera find their bearings by vision, smell and the other senses. The body colouring undoubtedly helps mutual recognition, so that they can distinguish each other from their surroundings [22], but what part does ultra-violet light play in this? Quite obviously objects which reflect identically will appear to lepidoptera to be of identical colours, i.e. indistinguishable if they reflect equal proportions of all colours from ultra-violet to red. Any change in the proportion of any of these colours inevitably changes the colour as perceived by the insect. A white object which absorbs ultra-violet rays will appear to the insect to have a colour "complementary to ultra-violet", if we may use such an expression. For differentiating an object from a background it is immaterial which reflects less ultra-violet or any other light. In either case the object—butterfly, flower, etc.—will be visible to the insect by the difference in colour against the background. In other words, the object, if it is to be visible, must stand

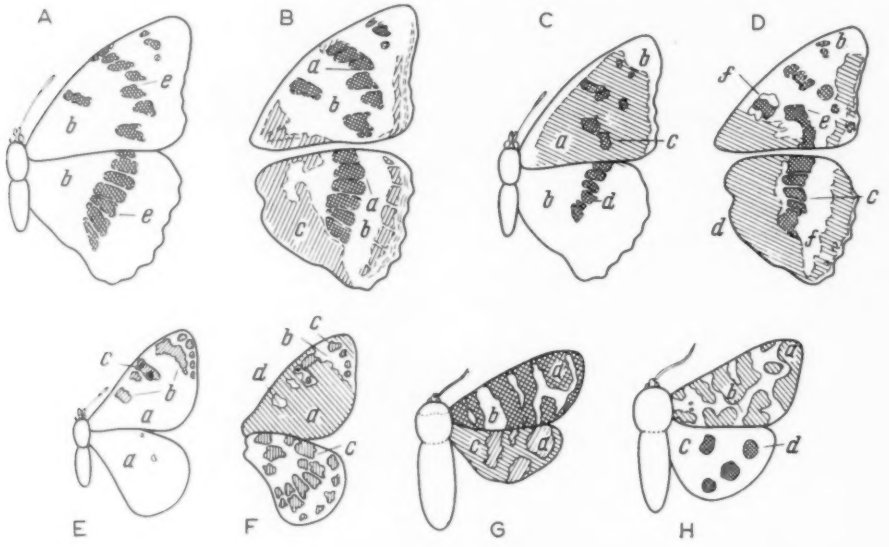

FIG. 5.

Males: A, B—*Limenitis populi* L. (from above and below)
C, D—*Apatura ilia* Schiff (from above and below)
E, F—*Hypermnestra helios* Nick (from above and below)
G—*Arctia hebe* L.
H—*A. caja* L.

out from its background [23]. An object is difficult to see, not when it reflects little light (we can see black objects clearly), but when it reflects light in the same way as its background.

Outcrops of chalk and limestone, sandy soil, the bark of trees, flowers, also clouds, blue sky and other environmental factors, are the background against which insects see each other; these reflect the ultra-violet fairly strongly. Against such backgrounds the body of an insect will, other things being equal, be the more noticeable the less it reflects ultra-violet. Lutz's [11] and Crane's [10] conclusions, viz. that ultra-violet light plays no part in the vision of insects, are therefore untenable, being based only on the fact that the insect body absorbs most of the ultra-violet. In their view ultra-

violet vision can be of adaptative importance to insects only if their body coverings reflect ultra-violet light strongly. In actual fact, ultra-violet vision is particularly suitable for differentiating structures which absorb strongly in the ultra-violet. The following example will make this point perfectly clear: yellow dyes are known to absorb only violet-blue light strongly. Hence in a yellow pattern seen against a white background in light containing no blue (e.g. through a yellow glass) not only will different shades of yellow be indistinguishable but the pattern itself will be invisible. Conversely all shades of yellow will be of maximum visibility when viewed in blue light. Similarly, a pattern of red material (absorbing green) will be most visible in green light and least in red.

The conclusion arrived at by these authors, viz. that ultra-violet vision of insects plays only a small part in their mutual recognition, is also refuted by the different ultra-violet reflection coefficients in different parts of butterfly wing patterns. The available data show that some patterns reflect ultra-violet rays strongly, other patterns weakly, thus producing a mixture of colours which is just what enables insects to recognize each other [24]. If the entire wing reflected ultra-violet equally strongly, as Lutz's and Crane's concepts would require, the colourings of butterflies would not appear varied to the insects and the value of ultra-violet vision would be completely nullified.

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CONCLUSIONS

The ultra-violet reflection coefficients (365 mμ) were determined photographically for the wing surfaces of male and female lepidoptera of more than fifty species. Maximum reflection (up to 40–70 per cent) was observed with the upper wing surfaces of yellow and orange *Gonepteryx*, *Catopsilia*, *Derca*, blue *Lycaena* and white *Nygmia*, *Spilosoma* and *Hepilaus*. In other species only certain parts of the wing pattern or androconia patches which cannot always be distinguished by their colour from the main wing surface reflect strongly.

Pronounced sexual differences in the ultra-violet reflection coefficients are characteristic of *Gonepteryx*, *Aporia*, *Lycaena* and some species of other genera. The male *G. rhamni L.*, for example, reflects about 40 per cent, the female only 7·5 per cent. This sexual dimorphism, while visible to the insect, is sometimes invisible to man, e.g. in male and female *A. crataegi L.*, which look very much alike.

The wings of certain butterflies (*Gonepteryx*, *Catopsilia*, *Aporia*), which appear single-hued to the human eye, have patterns which are visible only in ultra-violet light; these patterns are undoubtedly perceived by insect eyes, which are sensitive to ultra-violet.

The fact that butterflies have colour vision in the ultra-violet, while their wing surfaces and environmental factors (soil, stones, leaves, flowers) reflect ultra-violet rays in different degrees, seems to indicate that these rays are as much concerned in the butterfly's vision as longer-wave region of the spectrum. Crane's [10] conclusions, viz. that the ultra-violet plays no part in insect vision, are untenable and one-sided, ignoring, as they do, the importance of the background against which an object is seen. In a number of cases ultra-violet vision is of great advantage in differentiating objects that strongly absorb these rays, e.g. the integuments of insects.

Translated by O. S. WHITSTON

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OBSERVATIONS ON THE FLIGHT OF PRIONUS BEETLES AND MAYFLIES*

THE AERODYNAMICS OF INSECT FLIGHT

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(*Received* 16 *April* 1956)

As is well known, the fore-wings of beetles form rigid elytra which function primarily to protect the hind-wings and body while crawling. However, observations show that the elytra sometimes also function in the flight of the beetle.

There are various suggestions as to the role of the elytra in flight. As we have already pointed out [1], they are sometimes said to act as supporting surfaces, like the wings of an aeroplane. Indeed, the elytra are convex upwards and they could be lifted by the incident air-stream produced during flight, which would serve as an additional lifting force. Although this could give a perceptible effect when the wings are horizontal and completely motionless, when they are raised and vibrating, as usually happens in many beetles, the additional lifting force cannot be very significant. Therefore this unverified suggestion is open to doubt.

Magnan's [2] high-speed photographs showed that the elytra of many beetles are raised and lowered together with the hind-wings, but the amplitude of their vibrations

* *Biofizika*, **2**, No. 3, 369 [Reprint Order No. BIO 46].

is less. In the may-bug the hind-wings describe an arc of 130°, and the elytra 60°. The elytra only move vertically and do not vibrate around their longitudinal axes, but such vibrations would be very important in propelling the insect. In any case it is clear that the role of the elytra of beetles in flight is secondary and negligible, since the primary propulsive agent is the hind pair of webbed wings.

There are many beetles which fly with folded elytra, such as, for instance, the cetonids, in which, as is well known, the lateral edge of the elytra has an anterior groove through which the membranous wings emerge during flight. This shows that in many cases beetles can dispense with the elytra in flight. When the elytra were removed from various beetles [3, 4] species such as the cetonids were found to fly without the elytra, while others if deprived of them could not fly.

There is no doubt that in many beetles the elytra give the body transverse stability during flight. Many beetles, during flight, hold the elytra at a certain dihedral angle. The angle may vary: for instance, in female rhinoceros beetles it is 142° and therefore each makes an angle of 19° to the horizontal. But in many beetles the elytra are more raised, as in the prionus beetles where the angle is nearly 100°, giving approximately 40° with the horizontal.

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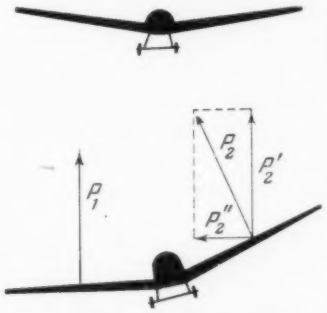

FIG. 1. Top: V-shaped arrangement of wings of an aeroplane. Bottom: aeroplane, tilted on its left wing, slips to the left, but returns to its normal position due to the V-shaped form of the wings.

The wings of an aeroplane are attached in such a way that seen from in front they form an oblique angle (like the letter V). Fig. 1 shows an aeroplane tilted over on its left wing. We will resolve the lift P_2 in its two components P_2' and P_2''. The component P_2'' is normal to the flight direction and causes an extra leftward velocity which in turn causes the aeroplane to slip leftwards, but with the longitudinal axis undeflected. This extra movement to the left causes a supplementary left-to-right air-stream which, flowing over the upper surface of the right wing, depresses it, whilst not affecting the left wing (P_1 being the lift), because the angle of attack is zero.* So a force acts on the rising right wing which turns the aeroplane around its longitudinal axis and returns it to its normal position.

There is some basis for suggesting that this V-shaped position of the elytra produces transverse stability and is indispensable for many beetles. This apparently also explains why many beetles deprived of their elytra start to turn over and fall, being deprived of that support in air which the elytra provide.

* The angle of attack is the angle at which the surface or wing meets the air-stream.

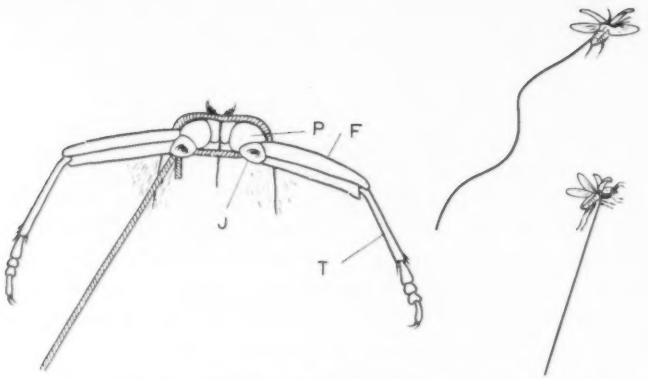

FIG. 2. Method of attaching threads to prionid beetles. *P*: pelvis, *J*: joint, *F*: femur, *T*: tarsus.

The author observed the flight of prionus beetles (*Prionus coriarius* L.) of the Cerambycidae family. The beetles were tied by threads passed around the pelvis of the middle pair of legs, as shown in Fig. 2, and were released on the threads which unwound from spools in almost free flight; this was as previously done in observations on the flight of dragonflies [5], and later on other insects (the fly *Echinomyia grossa* L., crane-fly *Tipula oleracea* L. and the wood-wasp *Sirex gigas* L.). The male prionids flew well and described large circles, oblique-angled zig-zags, or smooth curves. By gradually rewinding the thread it was possible to keep them flying along circular courses of radii about 1 m and thus to perform prolonged observations on the beetle in flight.

It was noticed that the V-shaped raised position of the elytra was constantly present and to a significant degree provided flight stability. If the elytra were flat and not bent at their upper ends, this effect would be less important because the dihedral angle is rather large (about 100°). Also the lower surfaces of the elytra are concave, scooping up a large mass of air, and are hooked at the ends, which accentuates this effect and contributes materially to the stability. Fig. 3 shows a beetle

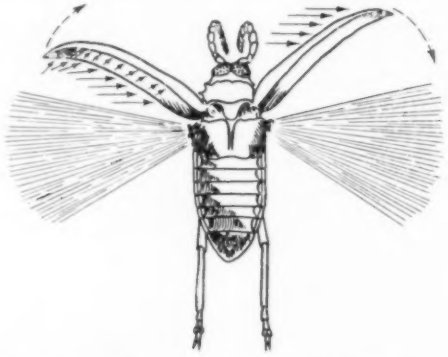

FIG. 3. Aerodynamic forces acting on the elytra, providing transverse stability. Plain arrows: direction of extra air-stream while tilting to the left. Arrows: direction of wing movement by which the extra air-stream corrects the tilt.

turning slightly to the left. The air flowing over the right elytrum from above and over the right hind-wing from below as a result of the list returns the beetle to its original position and at the same time maintains its axis horizontal. In practice the prionids fly all the time without listing in either direction; only the sharpest turns cause a very slight list. This maintains stability and there is no loss of manoeuvrability in flight, as we found. If we compare the flight of cetonids, which fold their elytra in flight (see above), we find that they tilt significantly while turning, which is related to their much greater manoeuvrability in flight as compared with prionids. Prionids which are deprived of their elytra do not fly, although they may sometimes make abortive attempts to do so.

Female prionids tied to threads do not always fly since they turn over on to their backs, apparently because many of them have more massive abdomens than the males, and are weighed down by the unlaid eggs, which affect their centres of gravity. For this reason it is impossible to tie them by the bases of the middle legs.

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By winding in the thread, it is possible to restrict the beetle to a circuit of 30–40 cm radius which is described rapidly, and is sometimes horizontal, sometimes vertical. In describing a vertical circuit the beetle passes through the lower part on its back, which does not occur in nature. This is due to the centrifugal force on the beetle.

When the radius is large enough and when the thread is unwound continuously or the observer follows the beetle on a long thread, the flight is perfectly free and normal, and as under natural conditions the insect sometimes changes its direction, making all possible turns, when the light thread, rapidly unwound, and freely pulled by the beetle, describes its track in the air.

Apparently the weight of the thread stimulates flight. If a sitting beetle is pulled up on the thread, it at once opens its elytra and, spreading its wings, flies off. This may be repeated many times, evoking flight at will. Prionids usually fly in the evenings, in twilight, but will fly by day when wakened by suspension. Suspension also stimulates flight in the other insects, which could be observed in tethered flight (see above), but it was particularly marked in prionids.

The stimulus to flight on a thread is apparently related to the so-called "tarsal reflex", in which the flight movement of the wings is evoked by reflex in response to the loss of contact between the extremities and the surfaces which they were touching, and which was used by Williams and Chadwick [6] in their experiments. The sensory receptors involved are in the feet.

In flight the prionus beetle inclines its body at an angle of 45° to the horizontal, the forepart being raised, the legs extended forwards and the feet, as it were, groping in the air (Fig. 4). It was noticed that if the forefeet were damaged flight did not take place at all and there were only feeble attempts to fly. Removal of the feet from the middle- and hind-legs likewise affects flight, which worsens, does not last long, is unwilling, and in some cases can only be evoked artificially by suspending the beetle on a thread.

The underside of the first four joints of the feet of the prionid are thickly covered with small hairs (Fig. 5), which could possibly be tactile. If this were so then it would be understandable that injury and removal of the feet should affect flight, because the groping feet, meeting the current of air, might be significant for the normal position of flight, the maintenance of the correct direction of the plane of vibration

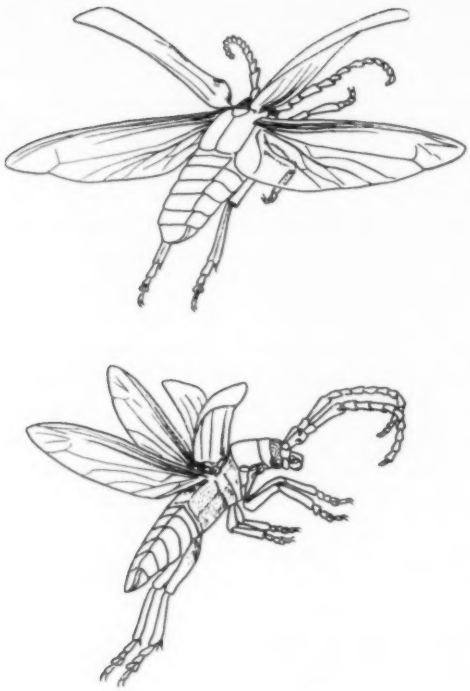

FIG. 4. Prionid beetles in flight.

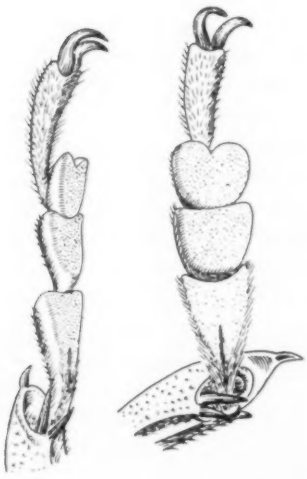

FIG. 5. The foot of a prionid with hairs on the first four joints. Left: half-turned view. Right: frontal view.

of the wings and the orientation of the body in flight. This suggestion is a subject for observation and confirmation.

The extended forefeet in male mayflies also appear sensitive. At first sight they seem to replace the very short antennae of these insects and to be extended instead of them. Mayflies have a fairly simple up-and-down wing motion, with only slight variations in the angle of attack.

The author observed the flight during the swarming or "dance" of mayflies, in which these tactile feet are extended forwards and, meeting the faint breeze, orient the insect to it. The observations were made principally on the Danish mayfly, *Ephemera danica* Müll.

The swarming of mayflies is a well-known phenomenon. Sometimes small groups, but more frequently large numbers of males, tumble about in the air, each insect rocketing upwards, and then parachuting, descending on its wings, which are spread at about 120°. The "dancing" individuals all face the same direction, and even in apparently perfect calm it may easily be observed that the head and forward-extended long forelegs are always directed against the air current, whether this be a faint breeze or a more perceptible wind.

The females have shorter forelegs and do not take part in the "dance", i.e. in the parachuting and upwards rocketing movement, but they fly into the dancing swarm always in the same way, orienting against the wind, and the males seize them by their long forelegs, and fertilize them.

The insects fly inclined upwards, as shown in Figs. 6 and 7, to a height of 1 or 2 m, the tail threads hanging down pressed together. On reaching a certain height they cease moving their wings, raise them slightly to form a V-shape, the abdomen being

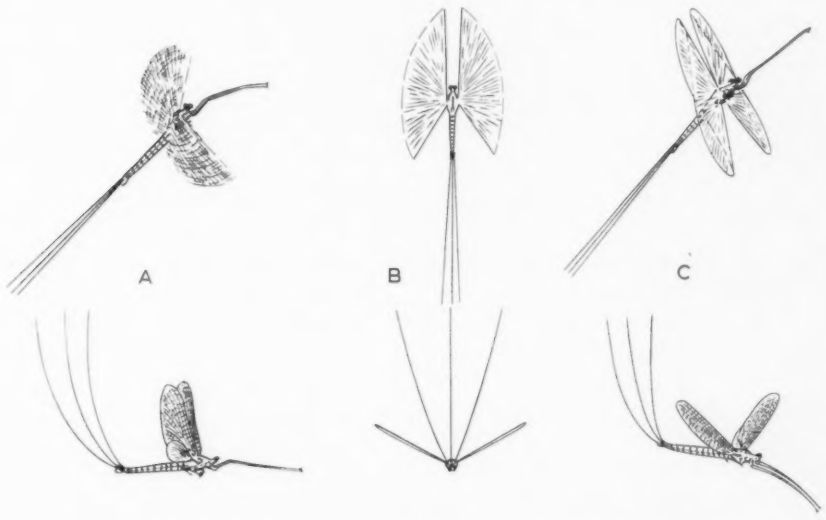

FIG. 6. Mayflies flying during the "dance". A: side view. B: dorsal view. C: half-turned view. Top: upward flight on vibrating wings. Bottom: parachuting on motionless and V-shaped wings.

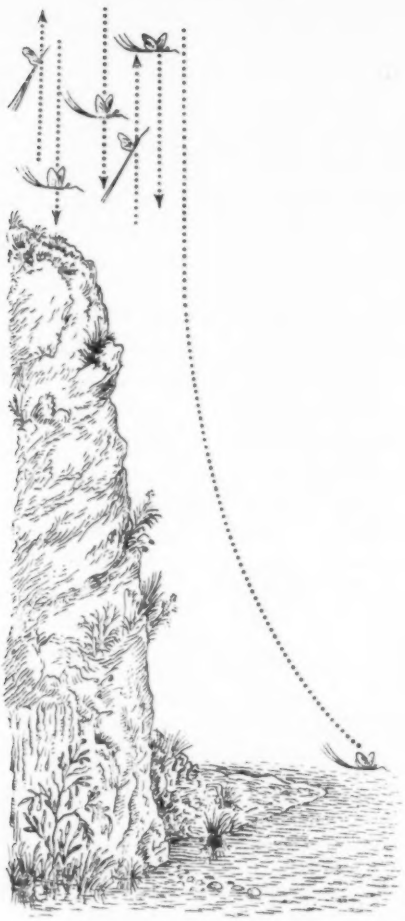

Fig. 7. Left: a swarm during the "dance". Right: mayflies parachuting from a great height. The descent is indicated by the dots.

slightly arched* upwards against the wind, the tail threads being spread out into the air to facilitate parachuting (Figs. 6 and 8).

Not even a fairly strong headwind can carry the mayflies backwards: they rise quite vertically (Fig. 7). Apparently the air-flow caused by the flight streams over the wrinkles on the lower surfaces of the crimped wings and impels the insect forwards. In this way the insect overcomes the frontal resistance caused by the wind, being helped by the currents flowing from the widely extended tail threads, through the movements of which the insect can to some extent regulate the strength of the flow (Fig. 8). The V-shaped extended wings, as in the beetles, facilitate stability in face of a changing wind direction. In lengthy parachuting by mayflies from great heights down a river bank the lower regions of the tracks deviated from vertical and even

*As is well known, the stomachs and intestines of mayflies are full of air.

described arcs forwards against the wind because the force of reaction exceeded the wind pressure (i.e. the frontal resistance).

To confirm these suggestions, models roughly resembling mayflies were prepared. In some the wings were flat, in others they were crimped, the wrinkles fanning out backwards (Fig. 8). The tail threads of such models were slightly bent upwards. Thrown from a certain height in calm conditions, the models with flat

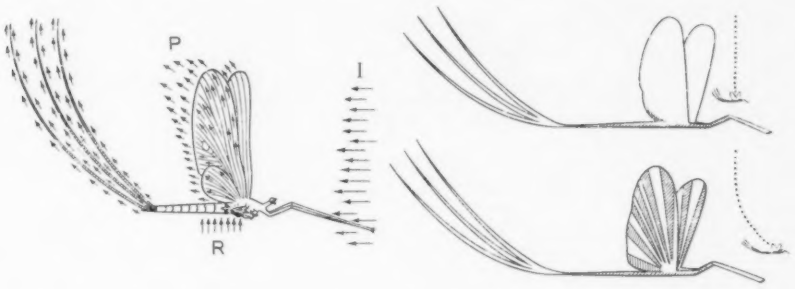

FIG. 8. Left: a parachuting mayfly. Right: paper models with plain and crimped wings plus descending trajectories. The arrows show the direction of the air-currents close to the lower surface of the wings and tail threads. *I*: incident air-stream. *R*: resistance of air lying under the descending insect. *P*: propulsive air-stream.

wings descended slowly, parachuting quite vertically, those with crimped wings parachuted and floated slightly forwards (Fig. 8), which indicated a reaction impelling the models forwards.

In perfect calm mayflies do not "dance". The "dance" always indicates the existence of some slight breeze or local air current.

Later, a similar orientation against the wind of all the individuals in a swarm was observed in other insects, for instance, in chironomids, which also "dance" strongly up and down when swarming.

The orientation of insects against the wind has also been reported in the literature. Thus Shvanvich [7] describes certain sensory hairs on the head of the desert locust (*Schistocerca gregaria transiens*). Some of these, according to Weiss-Fogh [8], stimulate flight in the direction of the air-stream, others re-establish it on disturbance and act as horizontal stabilizers.

In the insects examined here the legs are extended in flight, being even slightly apart in the beetles. However, in fast very manoeuvrable insects, such as hover-flies (Syrphidae), the position of the legs in flight is streamlined. Their legs are fairly flattened against the body.

CONCLUSIONS

To sum up we conclude as follows:

(1) In some beetles the elytra provide transverse stability in flight.

(2) The intactness of the hairy feet of prionids with which they sense the oncoming air current (just as the desert locust does with the sensory hairs on its head) is important for flight.

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(3) The parachuting flight of mayflies, orientated against the wind, is not a simple parachuting, but a more complicated process, in which the employment of reactive aerodynamic forces produced by the air-flow over the wings and tail threads, plays a part. These forces balance the frontal wind resistance.

It is interesting that suspension of insects in the air stimulates flight via the "tarsal reflex".

Translated by L. BIRKETT

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THE USE OF ULTRA-VIOLET LIGHT IN DIAGNOSING INTERNAL FUNGAL INFECTIONS IN SEEDS OF AGRICULTURAL CROPS*

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(*Received* 18 *December* 1955)

THE effectiveness of ultra-violet light in uniquely specifying diverse organic and inorganic substances opens up new vistas when this physical method is used in phytopathological investigations on seeds. Many parasitic infections of agricultural crops which cause crop losses and lowering of seed quality are transmitted through seeds. Special phytopathological methods have been worked out for detecting hidden internal infections in seeds. However, even the simplest of these are either laborious or involve lengthy analyses. It is particularly difficult to detect smut (*Ustilago tritici*) in the grain and to isolate it. In agricultural practice smut is still detected empirically from the presence of damaged ears during field-tests on the seeds.

Among the original methods for studying *Ustilago tritici* infections of wheat grains evolved in the U.S.S.R. between 1935 and 1939 (Budrina, Bubentsov, Skvortsov, Yablokova, Shvarts and Saburova) must be particularly noted the work of Yablokova, who introduced the use of fluorescence microscopy for differentiating fungal mycelia from grain tissues. The next stage in simplifying the method should be to use ultra-violet light for macroanalysis of infected grain, particularly since central seed-control laboratories possess suitable equipment, viz. mercury-quartz lamps with Uviol glass. The results of some exploratory experiments on the

* *Biofizika*, **2**, No. 3, 376 [Reprint Order No.: BIO 47].

macroanalysis of infected wheat grains using filtered ultra-violet light (mercury-quartz lamp LIUM-1) are given below.

Two batches of Lutescens Red Fife wheat grain were used: one collected from ears artificially infected with the fungus in the flowering stage, and one non-infected, to serve as control. The non-infected grains began to fluoresce with a blue light a few seconds after being exposed to ultra-violet light. The artificially infected grains did not fluoresce and appeared dark and dull. For further confirmation, these two batches of grain were sown in an experimental plot. The non-infected (fluorescing) grains produced healthy plants; in the area sown with the infected (non-fluorescing) seeds, 68 per cent of the plants produced were infected. The fact that smut did not appear in 100 per cent of the plants may be due to the peculiarities of the plant–parasite interrelationship, when the fungus sometimes cannot reach the growing point and the plant escapes infection.

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Healthy seeds of Tulun-7013/8 wheat (fluorescing blue) were sown in an experimental plot. At the beginning of ear-formation—15–25 July—the plants were artificially infected. The spores were introduced by means of forceps, separating the floral scales. After the day of inoculation single ears were cut down at 10 day intervals and the seeds subjected to fluorescence analysis. In the milky phase of ripening no difference was observed between the artificially infected and the control grains—both fluoresced weakly. In the waxy phase of ripening the infected grains were harder and had a darker integument. Differences in the character of the fluorescence developed during ripening. The control seeds fluoresced evenly, with a blue light; the infected ones fluoresced unevenly, in discrete points. Evidently only ripe (dry) grains should be used for analysis.

The observed peculiarity of seeds, namely, the absence of fluorescence at the beginning of ripening, and its development later, accords with theoretical explanations appearing in the literature. The less the water content and the greater the hardness of tissue, the stronger the fluorescence, and vice versa. Complete or partial disappearance of fluorescence in infected grains, which was revealed in the experiments, points to the fungus having reached a definite stage of development; this had, as may be expected, caused changes in the inner structure of the infected grain resulting in metabolic impairment.

Further experiments have shown that the fluorescence quenching caused by *Ustilago tritici* is not specific to this fungus. Thus seeds of low sprouting capacity, with or without saprophitic microflora, also do not fluoresce. Quenching of fluorescence may be caused by other parasitic infections, e.g. *Fusarium* or *Helminthosporium* fungi, and by bacteria.

Seeds of other agricultural crops behave to some extent analogously. Healthy pea seeds fluoresce with a bluish-green light, but areas infected with *Ascochyta* or *Fusarium* fungi appear as dull, brown-red spots. Seeds with a dark integument, such as beetroot, or cruciferous vegetables, do not fluoresce. But an interesting phenomenon was revealed when round beet seeds infected with the *Phoma* fungus were studied. Points of dull white luminescence appeared on the dark surface of these, corresponding to the distribution of the surface fructification bodies of the fungus. Since the pycnidium is a dark-coloured body, fluorescence is usually due to its contents —the colourless spores showing through the mouth of the pycnidium.

CONCLUSIONS

Preliminary experiments on the use of ultra-violet light in analysis of infected seeds opens up prospects of introducing fluorescence methods into phytopathological seed investigation practice.

So far the use of the method is confined to seeds with a light integument. Normal healthy seeds of wheat, peas and soya beans fluoresce in filtered ultra-violet light. Pathological conditions caused by internal fungal or bacterial infections quench the fluorescence. This phenomenon is also seen in unripe, non-sprouting, or very moist seeds.

Thus fluorescence analysis of seeds may be recommended for seed-control laboratories, selection stations, etc. as a quick and accurate method of checking the quality of the seeds.

Translated by I. GORE

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THE ROLE OF THE NERVOUS SYSTEM IN REACTIONS TO UHF ELECTROMAGNETIC FIELDS*

N. N. LIVSHITS

(*Received* 15 *November* 1956)

IN order to understand the mechanism of the action of physical factors on the complex animal organism it is important to decide the role played in the observed effects by direct local action on the cells, tissues and organs and of the degree of influence of the reaction of the entire organism, as accomplished by nervous regulation. Of particular interest from this point of view are the physical factors of penetrating action, among which may be counted electromagnetic fields of ultra high frequency (UHF).

The biological action of UHF was intensively studied in the 'thirties of the present century, but after the Second World War the attention of workers was attracted to the action of other types of energy (ionizing radiations, and to a lesser extent ultrasonics). In the world literature of the post-war period there are very few papers on the reactions of organisms to UHF.

The experimental material on the role of the nervous system in reactions to UHF, accumulated in the literature, was not fully generalized, while the accumulation of this material has not only not decreased, but greatly increased in our time, because the use of UHF increases annually in various fields of technology. Apart from this, the problem is of fundamental importance as part of the large problem of the action of physical factors on complex organisms, the study of which is continually attracting more attention.

For this reason we think it expedient to publish this review, in spite of the fact that little has been published on the subject recently.

Numerous experimental investigations have established the fact that the action of UHF evokes changes in many physiological functions [1–3, etc.]; amongst these are the direct influence of UHF on organs and tissues, the chemistry of the blood, enzyme processes and also on the reaction of the organism, accomplished by means

* *Biofizika*, **2**, No. 3, 378 [Reprint Order No.: BIO 48].

of nervous regulation, both pathological and compensatory. The explanation of the role of the nervous system in this complex of phenomena is very involved; for this purpose one may use the following materials in the literature:

(1) Comparison of the action of UHF on denervated organs with its action on organs with intact innervations.

(2) Investigation of the action of UHF in conjunction with pharmacological substances, with selective action on the particular section of the nervous system.

(3) Observation of the specificity of the reactions to the effect of UHF and comparison of them with well-studied specific reactions to stimuli or to the depression of definite parts of the nervous system.

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One of the first reactions of the organism in UHF fields to be discovered was the rise in temperature. The cause of the rise in temperature could be a simple warming up of the body as an imperfect conductor in the UHF field and direct changes in the oxidative processes in the tissues and organs under the influence of UHF. Besides this, in temperature reactions a large part might be played by reflex changes in heat production and heat loss, and also by the direct influence of the UHF field on the nervous centres. Hence the complexity of the analysis of even such a relatively simple reaction as the temperature effect in the response of the organism to the influence of UHF fields. Many facts indicate that this is not just a simple warming up of the body in the UHF field. The most noticeable of these is that certain actions of UHF evoke not a rise in temperature but a fall (which can be explained as hypercompensation), and that only after increasing the dosage of UHF does the temperature start rising [4–6].

In experiments on guinea-pigs it was shown that the warming of an animal object is proportional not to the square, but to a higher power of the strength of the UHF field, and in this respect the dependence of the heat effect on the acting physical energy varies with the strength of the field.

In passing from 310 to 440V the warming of the object is proportional to the field strength to the power 3·7, but from 440 to 640V the power is 2·5, because a UHF field of such strength causes a suppression of the catabolic processes [5].

Living organisms in UHF fields warm up more in the majority of cases than the corpses of animals of the same weight [7]. The specificity of the warming up of living organisms in UHF fields is indicated by the individuality of the temperature reactions found among animals and humans. Under the action of UHF fields on the abdomens of rabbits, in one case the temperature rose to 45°, in another it did not exceed 42°. In humans under identical conditions of the action of UHF fields on the lateral body surface the temperature fluctuated in various individuals from 38° to 41° [6]. Individual fluctuations in the temperature reactions are noted by all authors who investigated the temperature of animals or humans in UHF fields [8–10]. In the same individual animal the temperature reaction varies with repeated actions, and the phenomenon of "habituation" develops, in which "powerful fields, used after weaker fields, may cause even lesser effect" [5].

The temperature reaction varies substantially after destruction of the completeness of the nervous system. In rabbits in which the spinal cord has been cut between

the sixth and seventh cervical segments the rise in temperature under the action of UHF was half that in controls [7]. Section of the cervical sympathetic nerves or destruction of the cervical sympathetic ganglia in experiments on cats and on rabbits also reduced the rise in temperature under the general action of UHF [11].

The rise in temperature in UHF fields is partly connected with the rise in the basic exchange. Tonkikh [12] found in cats, under the influence of a UHF field on the head, a rise in temperature of 1·4°, invariably accompanied by an increase in the basal metabolic rate. After bilateral destruction of the star-shaped and lower cervical sympathetic ganglia, and also section of the abdominal nerves, there was no increase in the basal metabolic rate in UHF field, and the rise in temperature was less marked than in cats with undamaged nervous systems.

The nervous system plays a great role also in the increase in the basal metabolic rate under the general action of an UHF field [13]. In frogs, decerebrated at the upper limit of the medulla oblongata, the rise in the general metabolism in an UHF field is less than in frogs with uninjured brains. Against a background of adremalization and nicotinization, which evoke a rise in the excitability of the sympathetic nervous system, the UHF field acts more sharply on the metabolism, but after the introduction of ginergen, which depresses the sympathetic nervous system, the increase in the basal metabolism was less than in the control animals.

Hyperthermia in animals in a UHF field is to a great degree dependent upon increased heat production due to the mobilization of glycogen from the liver. It was established that the livers of mice, exposed to the action of a strong UHF field, contained 50–70 per cent less glycogen than the livers of the control animals [14]. In cats with denervated livers the temperature reaction in a UHF field was weak. Before the operation the rise in temperature was 1·1°, after denervation it was only 0·5°. The glycogen contents of the livers of these animals were lowered to a lesser degree than in intact animals [15]. The mobilization of glycogen from the liver results in hyperglycaemia.

It was shown that after denervation of the suprarenals the course of the sugar curve in a UHF field is substantially altered. Of nine rabbits with denervated suprarenals Eliseeva [16] found hypoglycaemia in five, an insignificant degree of hyperglycaemia in two and normal hyperglycaemia in only two. Eliseeva considers that after the exclusion of the suprarenals the action of the insulin apparatus begins to prevail.

The thermoregulatory centres react to the rising temperature by increasing the heat-exchange. The great significance of the processes of thermoregulation in the thermo-effects of the UHF field is indicated by the fact that in epilated rabbits the rise in body temperature in a UHF field is only slightly reduced, while amputation of the ears increases it sharply [17].

Many authors note an increase in the heat loss frequently commencing in a UHF field even before rise in temperature [6, 7]. Glezer and Debevinskii [18] in experiments conducted upon themselves, under the general influence of UHF found a copious perspiration, while the temperature inside the mouth rose only to 37·5°, or to a maximum of 38·6°.

The excitation of the thermoregulatory centres in a UHF field is so great that it sometimes evokes dominance in them. The phenomenon was found by Glezer [19]

in one of the dogs in an experiment on the influence of the action of UHF on the salivary feeding conditioned reflexes. This dog was exposed to the action of UHF which evoked a rise of temperature of 0·5°, and the activation of the phenomena connected with the heat-loss (more frequent breathing, protrusion of the tongue and so on). In the subsequent experiments, in which the UHF was not used, the dog responded to every conditioned feeding stimulus by reactions connected with an increase in heat-loss.

Very powerful influences lead to the destruction of thermoregulation, and then the temperature reaction of the animal under the influence of UHF is greatly changed.

Seguin and Gastelain [20] observed under the influence of desymmetrized waves, at doses which evoked a rise in temperature of 1° in a minute, live mice warmed up less quickly than the corpses of mice of the same weight. Under more powerful fields not only the warming, but also the cooling of both live and dead mice was equally rapid, and the live mice died when the temperature dropped to 27°. The death could be speeded up by heating. Apparently the influence used in these experiments, completely destroyed the thermoregulation.

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The role of the nervous system in reactions of the organism is very sharply brought out in the analysis of the action of a UHF field on the cardiac-vascular system. Pflomm [21] observed vagomimetic action of a UHF field on the frog's heart, while there was no such phenomenon on atropinized animals. On the basis of these observations and also of experiments on the action of UHF fields on blood-vessels (we discuss these experiments later) the author concluded that the UHF field stimulates the parasympathetic section of the nervous system and inhibits the sympathetic. This situation is true only of the particular case observed by the authors. In actual fact, a UHF field may affect the heart and vagomimetic and sympaticomimetic actions, and not infrequently both these effects may be found at the same time in the most varied combinations.

The complexity of the combination of vagomimetic and sympaticomimetic effects under the influence of UHF fields is clear from experiments not only on the entire organism, but also upon the isolated heart. In the latter case these phenomena may be explained as the action of UHF on the heart's own regulating centres and the peripheral ends of the centrifugal nerves. The literature contains mention of speeding-up of the rhythm and the contemporaneous increase in the amplitude of the heart contractions under the influence of UHF [6, 22, 23], of slowing in the rhythm with decrease in the amplitude [6, 22, 24], of speeding-up of the rhythm without change in the amplitude [6] and speeding-up of the rhythm with decrease in the amplitude [22, 30]. Apart from this, in experiments using frogs' hearts under the influence of UHF, the correlation of the rhythm of the auricles and ventricle was found to be upset, which indicates the destruction of the functions of the heart's own regulatory centres [22, 31–33].

Lebedinskii in 1937, on the basis of the material then available, remarked that "phenomena on the part of the heart are completely explained from the aspect of factually proven phenomena of excitation with subsequent fall in the vegetative nervous apparatus and of atypical muscular tissues" [34, p. 126]. In fact experiments based on the uses of various methods, confirm the leading role of the nervous system and changes of cardiac action, caused by the influence of UHF.

Lavrentieva and Fedorov [35] showed that in the frog's heart, extended according to Gramenitsky's method under the influence of UHF, at the same time as the rhythm of the heart contractions is slowed down there is a structural alteration in the synapses of the vagus nerve. When, after prolonged exposure, the alterations in the synapses have become irreversible, the negative chronotropic action of the UHF field ceases.

In frogs after denervation of the heart, or else smearing of the star ganglia with nicotine, the positive chronotropic effect under the influence of a UHF field is weaker than in animals with undamaged innervation of the heart [22]. In cats after destruction of the star ganglia the rhythm of heart contractions lags behind the rise in temperature, while in control animals there is a corresponding chronotropic and temperature effect [18]. It is interesting that the lagging of the increase in pulse rate behind the rise in temperature in a UHF field is also observed in certain persons [8].

For the understanding of the role of the cardiac nerves and the reactions of the heart to the influence of UHF, the experiments on the influence of vegetable poisons on the reactivity of the heart to UHF are important. It may be said to be firmly established that treatment of the heart with atropine hinders the slowing down of the heart contractions under the influence of UHF. Some authors have registered complete cessation of the negative chronotropic effect of the UHF field in the atropinized heart [21, 22, 36], and others a partial cessation [7, 37]. Muscarin, which increases the tone of the vagus nerve, hinders the development of a positive chronotropic effect of the UHF field [29].

The influence of a UHF field extends the post-action of histamine in experiments on isolated rabbit hearts [6]. The preliminary treatment of the isolated rabbit heart with histamine sharply increases the positive inotropic effect of the influence of UHF, causing only a slight increase in the amplitude of the heart contractions [38] of control preparations. There is some indication that the influence of adrenalin on the heart function is weakened by UHF. A mixture of adrenalin and acetylcholine, affecting a normal heart in the same way as adrenalin, acts on a heart under the influence of UHF at first like acetylcholine, and only later like adrenalin [36]. But in some cases the UHF field does not weaken the action of adrenalin, but strengthens it. Militsin [40] observed a sensitization of the isolated rabbit heart to adrenalin under the influence of UHF. The effect was particularly marked on a fatigued heart.

Thus, the experiments in which the influence of UHF and vegetable poisons was combined, verify the fact that a UHF field alters the sensitivity of the peripheral cardiac nerves and their termini. This conclusion is also obtained from experiments in which other methods were used.

A marked rise in the sensitivity of the vagus nerve in a UHF field was shown in the very convincing experiment by Glezer [22]. Under the general influence of a UHF field on a frog, in the period when the positive chronotropic effect is giving place to a negative one, simply touching the abdominal skin with a glass rod causes a slowing down, and often a cessation of the heart contractions. Under usual conditions an energetic tapping of the abdominal wall is needed for a similar effect.

Later Morellini and Ingrav [41] also observed a rise in the sensitivity of the vagus nerve under the action of UHF. Their experiments were conducted on 22 subjects (both fit and unfit), and the influence of UHF on the optico-cardiac reflexes and arterial pressure was studied by them.

Vasilenko [26] showed that, after the influence of a UHF field, stimulation of both the sympathetic and the vagus nerves causes a more marked effect than normally. The author explains the facts which he observed by saying that the influence of the UHF field raises the sensitivity of the heart to both nerves. But it is possible that there is also in this case a raising of the sensitivity of the centripetal nerves themselves. However, the author is correct in asserting that the possibility of a raising of the sensitivity of the heart muscles under the influence of a UHF field must be taken into account. It is established that the rheobase and chronaxis of the heart under spontaneous stimulation of the exterior of the abdomen may be either lowered or raised in a UHF field [8, 42].

Changes in the diameters of blood vessels in UHF fields are very graphic. At present there are enough demonstrations of the neurogenic mechanism of this reaction. Pflomm [21] observed that under the influence of UHF fields on the web of the frog there is for a short time a constriction of the vessels, giving place to a very sudden dilation of them. He regarded these experiments, and also those on the action of UHF fields on the heart, as conclusive evidence that in a UHF field there is an increase in the tonus of the parasympathetic section of the nervous system and a decrease in the tonus of the sympathetic section. This view seemed to be wrong in relation to the vessel innervation. It was established that the changes in the lumen of the vessels in a UHF field, as described by Pflomm, are by no means always observed. Under various forms of influence it has been observed that there is at first an expansion of the vessels, changing into a constriction, after which there sets in a maximal dilation [44] or else a prolonged constriction of the vessels in the second phase [45, 46]. It has been shown that the course of the vessel reaction to the UHF field depends upon the method of its influence. If a slow regular warming-up of the tissues is secured, then there is a dilation of the vessels, particularly capillaries. If the temperature of the tissues rises rapidly (1–2° and more per minute), there is a sharp contraction of the vessels, leading sometimes to a stoppage of the circulation [18].

Glezer [18], in experiments on frogs, showed that after section of the posterior roots, which carry the vessel-dilatory fibres to the vessels of the extremities, contraction of the vessels in a UHF field is possible, but the recovery of a normal lumen in the vessels after cessation of the influence is much slower than in normal cases. Nor does complete de-afferentation of the extremities exclude the possibility of a vessel-constricting effect of a UHF field, from which the author concluded that the reflex mechanism of this reaction is not the only one.

After section of the sympathetic tract, coming from the extremities of the frog, a general influence of UHF does not cause dilation of the vessels, but local influence does cause the constricting effect [18]. Therefore, constriction of the vessels under the general influence of the UHF field is effected via the nervous system. Under local action after section of the sympathetic nerves vessel constriction may be caused by spontaneous influence of the UHF field on the vessel wall or else on the peripheral terminations of the sympathetic nerves. The reaction of the vessels to stimulation of the sympathetic nervous system is sharply altered under the influence of a UHF field.

Under the influence of UHF on the rabbit's ear, stimulation of the cervical sympathetic ganglion with a faradic current causes not constriction but dilation of the vessel [46, 47]. Similarly, the constricting reaction of the vessels of the frog's

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web does not take place on stimulation of the sympathetic trunk with a faradic current [21].

Opinions differ on whether there is an alteration in the action of vessels to adrenalin under the influence of UHF. Pflomm [21] found that, after the influence of UHF, adrenalin does not cause constriction of the vessels. His experiments were sharply criticized by other investigators. Orlov [48] and Slavskii [6] showed that Pflomm used very powerful UHF fields, which causes irreversible changes in the vessel walls. Using UHF fields of lethal power, Orlov and Slavskii did not find an alteration in the reaction of the vessels to adrenalin.

Baranov [49], on the basis of experiments with the isolated rabbit's ear, came to the conclusion that the influence of the UHF field does not make the vessels refractory to adrenalin. Lob [50] came to the same conclusion. In his experiments, the constriction of the vessels in the frog's web, caused by the action of adrenalin solutions (at a dilution of 1: 1000), was not removed by prolonged influence to UHF. Lob, like Slavskii and Orlov, states that lethal doses of UHF cause an irreversible alteration of the vessel wall which might lower its reactivity to adrenalin.

There is no doubt, however, that a decisive role in these experiments is played by the relation between the doses of adrenalin and the strength of the UHF field. Weiss, Pick and Tomberg [51], using adrenalin at a dilution of 1: 10,000, found that a UHF field caused dilation of the vessels in the frog's web, previously treated with adrenalin. Since treatment with warm physiological solution caused the same reactions, these authors, as also Lob, came to the conclusion that a UHF field does not specifically affect the tonus of the sympathetic innervation, but acts like ordinary heat. But Kirchev [46] showed in experiments on the isolated rabbit's ear, that after the influence of a UHF field the reaction of the vessels to adrenalin was weakened. Kirchev used small doses of UHF, acting for only short periods, and causing completely reversible changes in the lumen of the vessels, as may be clearly seen from the curves which he presents in his paper.

Kachkovskii [52], in experiments on humans, found no alteration in the reaction of the vessels of the forearm to adrenalin, introduced by means of iontophoresis. The reaction to histamine was weakened in 75 per cent of cases. This author used very small doses of UHF. The strength absorbed by the skin (measured according to Tatarinov's method) was approximately 1–4 W.

Owing to the contradictory material, it is impossible to decide whether the reaction of the vessels to adrenalin is altered by the influence of a UHF field.

There is not a great deal of material on the role of the nervous system in changes of blood pressure, caused by the influence of UHF. In intact animals, and animals with decerebrations or else high cuts of the spinal cord, it was not possible to observe any substantial difference in the reaction of the blood pressure to the influence of UHF [18].

The attempts to find an alteration (under the influence of UHF) in the reaction of the blood pressure on the introduction of adrenalin have likewise drawn negative results [7, 53].

A neurogenic mechanism of increasing the flow of blood under local influence of UHF was shown by the experiments of Lawrens *et al.* [54]. These authors found an increase in the rate of the circulation in the extremities of the dog under local influence

of a UHF field. After denervation of the extremities this reaction changed sharply. The rate of flow after influence of the UHF did not exceed the initial level, and further lowering of it took place more slowly than in the control experiment.

The investigations into the role of the nervous system in changes in diurase, caused by the influence of UHF, gave contradictory results.

In the work of Kozenko [55] there are sharp differences between the influence of UHF on denervated livers and livers with intact innervation. In chronic experiments, set up on dogs in which one side of the liver was denervated, it was established that while the completely innervated liver reached to the influence of UHF by a reduction of diurase, this reduction of diurase was never found in the denervated liver, and in fact it was increased in most cases. Filtration and reabsorption were in most cases increased, which was never noticed in the control liver.

Koiwa [56] investigated diuresis under the influence of UHF on a portion of the dog's kidney with complete kidney innervation, and in dogs after section of the sympathetic nerves, after section of the vagus nerve, and after complete denervation of the kidneys. In all cases the influence of the UHF field caused a reduction of diuresis. After section of the sympathetic nerves this effect was weaker, and after section of the vagus nerves it was stronger than in animals with intact innervation. Against a background of atropinization the influence of UHF on the processes of filtration and reabsorption was just as marked as after section of the vagus nerves. In animals with completely denervated kidneys the influence of UHF was weaker than in the controls, but still clearer than in animals with sectioned sympathetic nerves.

Koiwa explains these results as a vessel-dilatory action of UHF, which is more sharply seen in animals following section of the sympathetic nerve because in these cases after a drop in the vessel-dilatory action of the sympathetic nerve the vessels would be dilated before the influence. However, the possibility of removing the influence of UHF on the reflex anuria suggests an influence of UHF on the nervous regulation of diuresis [57]. Reflex anuria in these experiments was caused in dogs by mechanical stimulation with a glass rod on one of the ureters. The influence of UHF on a portion of the lining caused diuresis.

Local influence of UHF on a region of the stomach stimulates the secretion of the stomach juice. This was discovered in the experiments on dogs with the small intestine isolated according to Pavlov's method. The influence of UHF strengthened the secretion of histamine in the stomach [58]. An essential role in this reaction is played by changes in the nervous regulation of the secretion by the stomach; this is indicated by experiments in which the influence of UHF was combined with the introduction of neurotrophic poisons or with partial denervation of the small intestine.

The influence of UHF weakens, but does not completely stop, the inhibition of the stomach secretion caused by separate introduction of atropine, which excludes the parasympathetic innervation, or ergotamine, which acts similarly upon the sympathetic system—but if atropmine and ergotamine are injected together, the UHF does not stimulate the stomach secretion [59, 60]. After sectioning the junction through which pass the branches of the vagus nerve directed to the small intestine, the action of UHF on the stomach secretion is considerably weakened, and the increase in secretion of digestive juice under the influence of UHF is less strongly

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developed than before section. The stomach secretions are increased to a lesser degree in animals previously heated with atropine and ergotamine, than before section.

In experiments on isolated sections of the stomach of the rabbit, it was found that the influence of UHF weakens the reaction of the preparation to adrenaline and pilocarpine [6].

The assumption about a neurogenic mechanism for certain reactions of higher animals to the influence of UHF fields is supported also by some interesting experiments by Tkan and Prikhozhii [62], known to us only by some short references. These authors discovered that the influence on dogs of a UHF field causes a molecular alteration on the blood plasma. The same effect could be caused in dogs exposed to conditioned stimuli, previously combined with the influence of UHF.

The participation of the nervous system in reactions of the organism to the influence of UHF may take various paths. There is a possibility of a reflex mechanism, and also of changes in the nervous regulation under the spontaneous influence of UHF on the nervous system. Besides this, changes in the internal media of the organism may affect the functioning of the nervous system.

Many of the papers referred to above indicate the participation of a reflex mechanism on the biological effects of UHF. Changes in nervous regulation under the influence of UHF on nervous centres are also supported experimentally. Under the influence of UHF on the head the body temperature rises. This is connected with the influence of UHF on the thermoregulatory centres, because the UHF fields in these experiments were so weak and geometrically small that they could not cause a general rise in temperature [6]. In some cases the body temperature falls [6, 63].

A rise in temperature under the influence of UHF on the head is less marked than after destruction of both asteriated ganglia [64]. Stimulation of the thermoregulatory centres under the influence of UHF on the head causes a strong perspiration all over the body surface in humans [65, 66].

In rabbits subjected to the influence of powerful UHF on the occipital region, a feverish condition is found, followed by a sudden destruction of thermoregulation [67]. Under the influence of UHF on the head the basal metabolism rises [12, 68–71]. After the influence of a UHF field on the head hyperglycaemia is found [72–75], while in some cases the blood content of sugar is reduced [76]. There are also found changes in the blood content of residual nitrogen, cholesterol [77] and uric acid [78].

Under the influence of UHF on the cervical sympathetic ganglia the quantity of products of protein breakdown in the blood and skin increases [79] and there are changes in the metabolism of carbohydrate, fat and protein, in the cardiac muscle [80].

Under the influence of UHF on the head there is found a change in the rhythm of the heart contraction [19, 28, 81] and in the electrocardiogram [82].

Glezer [18] found that in frogs with intact brains the local influence of UHF on the head caused at first a speeding-up of the heart rhythm with an increase in amplitude, and later a slowing-down of the rhythm. With both hemispheres destroyed up to the thalmic region the effect was stronger in respect of a sharpening of the regulatory influence of the higher regions of the brain on the sympathetic centres. After destruction of the optic lobes, under influence of the remaining sections of the brain there was found only a slowing-down of the heart rhythm. Vessel reactions are found under the influence of UHF on the nervous centres. Glezer [18] found a sharp

constriction of the arteries and capillaries of the frog's web under the influence of UHF on the head. After section of the spinal cord this reaction did not occur. Under therapeutical action of UHF on the heads of humans there is found a dilation of the vessels of the base of the eye [7, 83, 84] and of the extremities [85].

Hyperaemia of the internal organs is found under local action of UHF on the cervico-thoracic segments of the spinal cord in rabbits [86].

The action of UHF on the head causes change in the blood pressure, both increasing it [18, 87] and decreasing it [76, 81, 83, 84].

In dogs with the brain cut above the anteriod border of the fourth ventricle, under the influence of UHF on the head there is found a wave-like fluctuation in the blood pressure [88].

Under the influence of UHF on the head there is a change in diuresis [65, 89], motor and secretory functions of the stomach [88, 89, 91] and the stimulation of erythropoiesis [92].

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Some authors have found, under local UHF action in the solar plexus region, an increase in the content of agglutin in the blood of previously vaccinated rabbits [93–95].

CONCLUSIONS

The study of the role of the nervous system in the reaction of a complex organism to the influence of fields of ultra high frequencies (UHF) has been very fruitful. It has been shown that the reaction of the organs and tissues to the influence of UHF are composed of local phenomena, caused primarily by the action of this factor on the cell and tissue, and the reaction of the organism, realized through the participation of nervous regulation.

Particular study has been made of temperature effects, for which in many cases it has been possible to determine the degree of participation of each of these mechanisms in the final result and to show that, under not too powerful influences the role of nervous regulation is decisive. A great deal of help in understanding the mechanism of the biological influence of UHF has come from the study of the role of the nervous system in the reactions of the cardio-vascular system. In spite of the occurrence of certain contradictions in the results of various investigations on separate problems, the leading significance of the nervous system in these reactions appears to be established, together with the indubitably large role of local action upon the walls of blood vessels, heart muscles and so on. There is evidence of the importance of nervous regulation in the reaction to the influence of UHF by organs of nutrition and secretion.

The participation of the nervous system in the reaction of the organism to the influence of UHF may take various paths. There is no doubt that the reflex mechanism plays a big role here. In this respect the possibility must be mentioned of two methods of realization of the reflex influence, as was established by Orbeli for the reaction of the organism to the action of ionizing radiations: "On one hand a spontaneous reflex on the stimulation of some organ or other, on the other hand a reflex on the organs of internal secretion, which alter the character of its functioning in a particular direction and secondarily affect the organism's whole system" [96, p. 8]. As an example of a reaction of the second type we have the discovery of hyperglycaemia under UHF, which is absent in the majority of animals with denervated extremities.

Together with the reflector mechanism, a significant role in the reactions of the organism to the action of UHF is played by spontaneous influence of UHF on various sections of the nervous system.

We have mentioned only a part of the experimental material which shows that the influence of UHF on the nervous centres causes changes in the function and tophism of organs and tissues. As a result of many investigations it is established that under the influence of UHF a significant functional dislocation takes place in all the links of the central and peripheral nervous systems.

Translated by L. BIRKETT

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BOOK REVIEW*

B. RAJEWSKY (*et al.*). Strahlendosis und Strahlenwirkung Tafeln und Erläuterungen Unterlagen für Strahlenschutr. Stuttgart, 2 Auflage, 1956

THE development of nuclear physics and the possibility of using atomic energy for the purposes of war and of peace have led to research on an enormous scale into the biological effects of radiation. In the last decade the authors of this monograph have set out to integrate, as far as possible, the often conflicting material available in the world literature and to produce an exhaustive bibliography on the development of radiation sickness, in mammals and in man, from the viewpoint of the dependence of radiation damage on the dose and physical conditions of irradiation, such as the dose-rate and the type of radiation during external irradiation, and on the amount and type of radioactive isotopes when administered internally.

The monograph consists of two main parts: (1) tables and graphs of radiation damage in relation to the dose and the conditions of irradiation, with explanatory

* *Biofizika*, **2**, No. 3, 390 [Reprint Order No.: BIO 49].

notes; (2) bibliography of the problems dealt with, including over 7000 titles covering about 10 years (1946–56).

The first part of the monograph contains the following sections:

1. Acute radiation sickness following short irradiations of animals and of man.

2. A résumé of data on the "time factor" in radiation damage: erythema, epilation, lethal mutations in *Drosophila*, lethal effects of prolonged and fractional irradiation in mice, effect of dose-rate on the average survival times of white mice after X-ray and fast neutron irradiation, effect on white mice of inhaling radium emanations in different concentrations in the air; the various components of natural radiation acting on man. The fact that the average survival time of mice increases on prolonged irradiation at extremely small neutron dose-rates (0·028–0·035 rad/day) is noteworthy from the point of view of protection tolerances.

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3. Comparisons of whole-body and local (head, trunk) irradiation of white mice. Dependence of skin erythema in man on conditions of irradiation. Doses received during X-ray diagnostic procedures in man. Effect of prolonged low-intensity irradiation (from 0·1 to 10 r per day) on the sex organs of mice, rats, rabbits, dogs. Effect of radiation on human foetuses. Comparative radio-sensitivity of different cells in animals.

4. Effect of radiation on blood and on blood-forming organs in animals and man on brief whole-body irradiation at 1000 r; prolonged irradiation at up to 0·1 r/day and at from 0·1 to 10 r/day; continuous irradiation with α-rays at various dose-rates; daily fast-neutron irradiation at varying dose levels.

5. Development of malignant tumours from short irradiations with β- and γ-rays and neutrons in mice, rats and rabbits, and from the administration of radioactive isotopes to animals.

6. Résumé of comparative data on genetic radiosensitivity in *Drosophila*, mice and man.

7. Relative biological effects of different types of radiation.

8. Permissible amounts of radioactive isotopes in the human organism and permissible concentrations of radioactive isotopes in drinking water and in air. The tables are compiled from the international recommendations of 1953. A table is given of water and air concentrations of some radioactive isotopes (^{32}P, ^{55}Fe, ^{64}Cu, ^{89}Sr, ^{90}Sr, ^{131}I, ^{140}Ba, ^{144}Ce) which on short-term administration (10 days) produce a dose of 30 r equiv. in the course of 50 years. Another is given of the permissible concentrations of fission products in water following an atomic explosion. This section includes a table of the content and metabolism of various elements in human organs and tissues and "standard" tables for man (weight of body and various organs, water and air metabolism). Table annotations give methods for calculating the permissible concentrations of radioactive isotopes in the human organism, in water and in air.

9. Radiation zones in atomic and hydrogen bomb explosions. Protection against 4·5 MeV γ-rays in an atomic explosion (water, concrete, earth, iron, lead). Long-lived isotopes in the atmosphere after an atomic explosion.

Thus, in spite of the seemingly limited number of problems dealt with by the

authors, the monograph is of interest to a wide circle of radiobiologists as a work of reference and a bibliography on the pathophysiology of radiation sickness and the toxicology of radioactive substances.

Two shortcomings of the book are the absence of a critical evaluation of the reliability of the material gathered, as a consequence of which a number of findings cited are chiefly of bibliographical value; and the complete absence of references to Soviet work.

IA. L. SHEKHTMAN

Translated by I. GORE

CURRENT EVENTS

THE SECOND ALL-UNION CONFERENCE ON PHOTOSYNTHESIS*

THE Second All-Union Conference on Photosynthesis took place in Moscow from 21 to 26 January 1957. The Conference was opened by Academician A. L. Kursanov who, in his inaugural address, remarked on the importance and achievements of the work being carried out, and emphasized that photosynthesis is one of those problems in which there is close contact between different branches of science—biology, chemistry and physics. And indeed, 129 papers were presented to the Conference, the authors of which included agrobiologists and agrochemists, plant physiologists and biochemists, microbiologists and geochemists, biophysicists and physicochemists, chemists and physicists. Many of the participants in the Conference and authors of papers had come from distant regions, from Siberia and Moldavia, Georgia and Azerbaijan, the Uzbek SSR, Ukraine and White Russia.

All this indicates the profound interest there is in the problem of photosynthesis in this country. At the same time the approach to the problem of photosynthesis on such a wide scientific basis has also determined the structure of the work of the Conference. The majority of the papers were heard and considered in the naturally subdivided sections, such as those of photochemistry, chloroplasts, the physiology of photosynthesis, composition of pigments, photosynthesis in water plants and micro-organisms, "products of photosynthesis", and the section on light-culture and research methods.

In a very short account, we concentrate attention on the most important of the biophysical problems, photochemistry and the composition of pigments, and limit our remarks on the work of the remaining sections to short annotations.

PHOTOCHEMISTRY

In the section on the photochemistry of pigments and the corresponding plenary session, aspects of the physics, spectroscopy, photochemistry and energetics of photosynthetic pigments and their analogues were considered.

Academician A. N. Terenin's paper on the physical phenomena underlying the primary photoreaction in chlorophyll was based on material obtained in his laboratory.

* *Biofizika*, **2**, No. 3, 392 [Reprint Order No. BIO 50].

The author examined the various primary ways in which the energy of a quantum of red light absorbed by a chlorophyll molecule may be transformed. The following energy transformations subsequent to excitation following absorption of a quantum are possible: (1) emission of fluorescence (with some Stokes shift); (2) radiationless deactivation, the excitation energy going over to energy of molecular vibration; (3) transition to a metastable state involving valence bond rupture—the biradical state of chlorophyll; (4) reversible physical or irreversible chemical reaction between the excited or metastable molecule and the substrate. The absorbed energy may be made available for photosynthesis in the last reaction.

The author dwells in detail on the lifetime of an excited chlorophyll molecule, since this determines the ultimate utilization of its energy. The lifetimes of excited molecules of chlorophyll and of its analogues were measured by the author's co-workers, O. D. Dmitrievskii and V. L. Ermolaev on A. M. Bonch-Bruevich's phase fluorimeter. It was found that the lifetime was only slightly dependent on the solvent, being shorter if the chlorophyll was bound to protein in living leaves. The lifetime was increased considerably by reducing the temperature to —180°C. Phosphorescence indicates that the metastable state is present; the phosphorescences of chlorophyll and other pigments were studied in detail in the author's laboratory.

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The reactivities of the excited and metastable states are of considerable interest. To elucidate these, experiments on the quenching (a) of the fluorescence and (b) of the phosphorescence of chlorophyll by ascorbic acid and phenylhydrazine, which cause reversible photoreduction, were carried out. Preliminary experiments had shown that phenylhydrazine was a more powerful quencher of phosphorescence and fluorescence than ascorbic acid.

Experiments with powerful light flashes, carried out in the author's laboratory, provide an alternative method of determining the lifetime and reactivity of the metastable state in chlorophyll.

A. N. Terenin, A. V. Kariakin and E. B. Liubomudrov showed that the absorption band in the red region was weakened or abolished by the light flashes because a large fraction of the molecules went over to the metastable state. The lifetimes of the colourless forms of Mg and Zn phthalocyanins were $\sim 0{\cdot}8 \times 10^{-3}$—$1 \times 10^{-3}$ sec at room temperature, and 4×10^{-3}—7×10^{-3} sec at the temperature of liquid air. Decolorization did not occur in the presence of oxygen, or with Cu, Fe and Co phthalocyanins. The reactivity of the metastable state can be assessed from its lifetime.

The concluding part of Terenin's paper dealt with energy and electron migration in pigments in the solid state. The author presented results which demonstrated that energy was not transferred between excited singlet molecules, but between the triplet levels of neighbouring pigment molecules (biradical exciton). Two types of reaction are possible: (1) migration of energy quanta via the biradical levels, and (2) migration of electron vacancies ("hole") via the lower levels. Energy migration within the colloid particles of chlorophyll has been confirmed in the author's laboratory by pigment sensitization of electron excitation in semiconductors. It was demonstrated that this consisted of the transfer of energy quanta from chlorophyll to the electrons in the semiconductor.

The author linked these results with the idea of a photosynthetic "unit" as an assembly of pigment molecules united solely for the purpose of energy migration.

To this the author added his own concepts of energy migration via the metastable levels and on hole migration.

A. A. Krasnovskii's paper dealt with the photobiochemical ways in which the pigments participate in photosynthesis. He dwelt in detail on the role of chlorophyll in the oxidation–reduction systems in photosynthesis, in the course of which an electron (proton) is transferred from water to CO_2 (the opposite of dark equilibrium), from the lower to the higher thermodynamic level.

Krasnovskii explained and demonstrated the direct participation of chlorophyll in photoreactions in which light energy is stored in the reaction products. Reversible pigment photoreductions are here fundamental; apart from chlorophyll itself, pigments with porphine, chlorine, bacteriochlorine and azoporphine structures are potentially suitable.

The author showed that absorption of a light quantum causes the following sequence of fundamental photoreduction processes in chlorophyll.

(1) Excitation to the triplet biradical state

$$X + h\nu \rightarrow X^* \rightarrow \cdot X\cdot$$

(2) Electron transfer from the donor, giving ion-radicals:

$$\cdot X\cdot + HA \rightarrow \cdot X^- + H^+A\cdot$$

(3) Proton transfer giving the photoreduced form of chlorophyll:

$$\cdot X^- + \cdot AH^+ \rightarrow \cdot XH + \cdot A$$

The author remarked that spectroscopic studies on living photosynthesizing organisms in the last few years have demonstrated absorption spectra for the cell pigments similar to that for photoreduced chlorophyll.

Chlorophyll photoreduction is connected, on the one hand, with the systems which oxidize water and liberate oxygen (chlorophyll), thereby receiving an electron from the reactants, and, on the other, with systems involving reduction of CO_2 (reduced chlorophyll donates an electron to the reactants thereby returning to its original state).

It has been shown that chlorophyll can receive protons from ascorbic acid, cysteine, iron-porphyrins, polyphenols, etc. in isolated systems, and that light-sensitized proton transfer to riboflavine, pyridine-nucleotide, etc. is possible. It is possible that the energy-rich reduced compounds so formed (pyridine-nucleotides and phosphate esters), may be used in the CO_2 reduction cycle.

Turning to the chloroplast granule pigments, the author noted that almost all the chlorophyll is polymerized, although some is in a monomeric, photosynthetically active state. Both these forms are bound to proteins and lipids, and are in labile equilibrium dependent on physiological conditions and the age of the plant. Attention was directed to the possible migration of energy between aggregated and monomeric pigment forms.

Concluding his paper, Krasnovskii proposed a photosynthesis scheme in which chlorophyll stores light energy by upgrading (in the thermodynamic sense) hydrogen (electrons); conjugation of the active photoproducts with the enzymes systems leads to CO_2 being taken up and oxygen being liberated from water.

V. B. Evstigneev, in his paper, dwelt on the photoreduction mechanism in chlorophyll. He demonstrated spectrophotometrically and electrometrically that

chlorophyll and its analogues are photoreduced in solution via extremely labile, potentially reactive, electrode-active, reduced pigment forms with free radical properties—ionized semiquinones. The author explained in detail how this reduced form develops in redox reactions in solutions, as sensitized by chlorophyll and its analogues; ultimately it reacts with a hydrogen (electron) acceptor.

G. P. Brin's paper on "A study of photo-oxidation sensitized by chlorophyll and pheophytin" presented data showing that ascorbic acid, cysteine, polyphenols, cytochrome *c* and a number of other compounds can be photo-oxidized in the presence of chlorophyll. The rate of photo-oxidation depends on the state of the chlorophyll in the solution: the activity was greatest when the dissolved chlorophyll was monomeric (in organic solvents and in aqueous solutions containing detergents). He considered that the hydrogen donor oxidations are linked to the reduction of chlorophyll.

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Bacteriochlorophyll can be reversibly photoreduced in the same way as chlorophyll (K. K. Voinovskii and A. A. Krasnovskii). Active, unstable, reversibly reacting photoproducts have been observed in the reversible photo-oxidation and reduction of chlorophyll. A characteristic feature is that the bacteriochlorophyll absorption spectrum in living photosynthetic bacteria agrees with that for the polymerized form of bacteriophyll. The view that aggregation and adsorption actually cause a red-shift in chlorophyll is also supported by Putseiko's finding sensitization of the photoeffect in oxide semiconductors by chlorophyll and its analogues. Putseiko found that chlorophyll and its derivatives, when adsorbed on zinc oxide and mercuric oxide, sensitize the photoelectric effects in these semiconductors markedly. The energy transfer from the pigment to electrons in traps near the surface, which liberates the electrons from these traps, is held to be the sensitization mechanism. The action spectrum for sensitized photoconduction agrees with the absorption spectrum of chlorophyll in dilute solution but is displaced 5–10 mμ towards the red.

The paper of N. I. Kobozev and I. M. Podgornii on "Determination of the magnetic susceptibility of chlorophyll adsorbed on aluminium hydroxide gel" dealt with the effect of adsorption on the physicochemical properties of chlorophyll. The authors found that crystalline diamagnetic chlorophyll becomes paramagnetic on adsorption. They showed that the curve of magnetic susceptibility vs. chlorophyll concentration has a sharp break where the layer passes from dimolecular to monomolecular.

Gachkovskii's paper, "Failure of the conception of the two forms of chlorophyll" (fluorescing and non-fluorescing) to conform with the spectra of living leaves met with serious objections during the discussions.

B. Ia. Dain and M. S. Ashkinaza dealt with "Photochemistry of iron derivatives of chlorophyll and the role of the intra-complex activation mechanism in photosynthesis", and intra-complex electron transfer during photosynthesis was examined. Oxidized complexes of iron-containing derivatives and analogues of chlorophyll with water, hydroxyl and other ions, were examined. On exposing these complexes to light the absorption bands characteristic of hydroxyl-complexes and aquo-complexes vanished and the bands of reduced complexes appeared. The authors suggest that the following processes are occurring: electron transfer from the chlorophyll nucleus to the central iron atom which is thus converted from Fe $\cdot\cdot\cdot$ to Fe $\cdot\cdot$, followed by electron transfer from the co-ordinated groups (water, hydroxyl, etc.) to the electron-deficient

chlorophyll nucleus. They suggest that the intra-complex activation mechanism, involving iron salts of the chloroplasts, may act as the factor ensuring the activation of water even in the first photochemical phase of photosynthesis.

In A. N. Sidorov's paper on "The effect of the central metal atom on chlorophyll as seen from its infra-red absorption spectra", the infra-red spectra of chlorophyll and the pheophytins of zinc, lead and trivalent iron are described. The spectra show that the central metal atom exercises a very real effect on the whole molecule, including the peripheral groups. The absorption band of the water associated with the pigment (3400 cm^{-1}) appears in solutions of chlorophyll + water in CCl_4, whereas pheophytin under the same conditions does not give this band; this is held to indicate that the Mg atom determines the reactivity of chlorophyll with water.

G. P. Gurinovich, E. N. Ermolenko, A. N. Sevchenko and K. N. Solov'ev dealt with the absorption spectra and luminescence of chlorophyll and metal derivatives of pheophytin. The absorption spectra showed that, unlike the case of pure pheophytin, the absorption maximum in the violet was displaced towards the red, and that in the red towards the violet for the complexes of pheophytin with $Zn^{\cdot\cdot}$, $Cu^{\cdot\cdot}$, $Cd^{\cdot\cdot}$, $Ag^{\cdot}$, $Ni^{\cdot\cdot}$, $Co^{\cdot\cdot}$ and $Sm^{\cdot\cdot\cdot}$. Luminescence in the red region was observed with $Zn^{\cdot\cdot}$, $Ni^{\cdot\cdot}$, $Cd^{\cdot\cdot}$ and $Co^{\cdot\cdot}$ but not for $Cu^{\cdot\cdot}$, $Ag^{\cdot}$ and $Hg^{\cdot}$ derivatives. The luminescence polarization of chlorophyll and its derivatives were examined, and every absorption band of chlorophyll and its derivatives, was shown to correspond with a particular electron transfer. The oscillator angles were also calculated, and the absorption and emission were shown to be of dipole type.

Fluorescence polarization and the relative quantum yield at various exciting wavelengths (S. V. Konev) were used to show that intramolecular migration of energy occurs in the photosynthetic pigment of red algae, phycoerythrin. Energy migration occurs in the phycoerythrin–chromoprotein, in which the protein carrier is combined with 10–12 chromophore groups: (1) between the chromophores within the chromoprotein and (2) between the protein part (tyrosine and phenylalanine) and the chromophore.

Iu. A. Vladimirov dealt with the same problem and gave particular attention to the mechanism of energy migration in the protein system. To clarify the mechanism of resonance of migration in the published experiments, synthetic dyes were used as energy donors in place of the aromatic groups of protein. The results show that energy migration occurs in the dye-carbohaemoglobin system by a resonance mechanism which leads to the dye sensitizing the photodissociation of CO from the haemoglobin.

G. A. Korsunovskii ("Photo-oxidation of water on heterogeneous sensitizers") described some analogue experiments on the oxidation of water on the surface of a semiconducting sensitizer. The hydroxyl radical was detected via the spectra of its secondary products formed by irradiating aqueous suspensions of zinc oxide, titanium dioxide and cadmium sulphide, with benzene, which is oxidized to phenol, as hydroxyl acceptor.

STATE OF PIGMENTS

Elucidation of the mechanism of photosynthesis and the part played by pigments requires detailed study of the states and transformations of pigments directly in the plant.

T. N. Godnev dealt with the theory of chlorphyll formation in plants. Experiments with labelled glucose indicated monosaccharides as a possible important chlorophyll precursor in etiolated leaves. The shift in the red absorption band of chlorophyll observed on reaction with protamine was assigned to the formation of a monomeric complex of chlorophylid with protein. The position of this absorption band can also be related to the absorption band of freshly formed pigment in the leaf.

This problem was examined in more detail by T. N. Godnev, A. A. Kravtsev and R. V. Efremov, who expressed the view that the freshly formed chlorophyll–protein complex must have the structure of a chlorophylid-protein.

The processes of chlorophyll formation in the plant were also discussed by Rubin. Observations on antibiotics, which suppress viridescence, and on certain enzyme poisons indicate that the biosynthesis of chlorophyll is linked with enzyme-catalysed oxidative processes.

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The composition of the chlorophyll in the chloroplasts was also considered by O. P. Osipova, who successively extracted the pigments and lipids from chloroplasts with polar and non-polar solvents. This method may make it possible to determine the relationship between the free chlorophyll (dissolved in the lipids) and the chlorophyll combined with protein in the chloroplasts.

T. G. Maslova and D. I. Sapozhnikov also investigated the composition of chlorophyll in leaves, the indicator selected being the heat-stable chlorophyll–protein complex which can be assessed by extracting the chlorophyll with a non-polar solvent after boiling the leaves.

The stability of the chlorophyll–lipoprotein linkage changes during growth. In the autumn period the link weakens, possibly because of a reduction in the amount of associated lipids. Chlorophyll composition in plants of various systematic groups was also investigated.

A. A. Shlyk reported his radiotracer studies of chlorophyll turnover. These experiments (on three plants), in his view, make it possible to assess the processes involved in the biosynthesis of chlorophylls *a* and *b*.

L. M. Vorob'eva described the photoreactions of an active form of chlorophyll in water–glycerine extracts from leaves. The presence of a rapidly decolorizing active form of chlorophyll together with stable aggregated forms was demonstrated, and it was possible to observe reversible photoreduction and photo-oxidation of chlorophyll in such "solutions" in their natural state. These experiments may serve as confirmation of the conception of reversible redox changes in chlorophyll *in vivo* during photosynthesis.

V. B. Evstigneev reported photo-electrochemical studies on the green suspensions obtained from whole-leaf homogenates. On exposing such "green solutions" to light absorbed only by chlorophyll the redox potential fell sharply, which may indicate the rapid formation of electrode-active reduced substances, and points to their having labile electrons and great reactivity. These substances are relatively stable under anaerobic conditions, and probably represent the transformation products of the photo-sensitive pigment-complex. The effects of temperature and inhibitors on the photo-electrochemical effect were also studied; the results indicated that enzyme systems related to the "oxidation" stage of photosynthesis were involved in giving active photoproducts.

The mechanism of the redox changes in chlorophyll during photosynthesis was dealt by V. M. Kutiurin. Using nitrogen isotopes it was shown that in *Elodea* chlorophyll biosynthesis is fairly slow and can occur in the dark. Only a very limited exchange with D_2O was observed during chlorophyll photosynthesis, indicating the absence of reversible dehydrogenation of chlorophyll during sensitization. Methods of investigating the composition and transformations of pigments in plants which retain the undisturbed structure and vital activity of the living leaf are particularly important. In this respect Bell's work on reversible spectral changes in plant leaves are of interest. Here, exposure of the living leaf to light produced a slight change in the absorption spectra. The magnitudes of the absorption coefficient changes depend on the durations of illumination and of the preceding dark period. Two simultaneous processes with different speeds, and differing in the character of their spectrum changes were observed.

A number of papers dealt with the changes in pigments and their relationships caused by various factors (particularly light). D. I. Sapozhnikov, T. A. Krasovskaia and A. N. Maevskaia reported their investigations on the light-induced changes in the ratios of the principal carotenoids in green leaves. The leaves were cooled rapidly to —78°C immediately after illumination to ensure quantitative determinations of the main carotenoids present. A regular differential change in the contents of lutein and biolaxantin was observed. This difference increased with intensity of illumination and disappeared in the dark. The view was expressed that enzyme systems regulate the relative levels of lutein and biolaxantin.

I. A. Chernavina studied the actions of various radiations on the biosynthesis and states of pigments in plants. The experiments indicated that red light produced a more intense accumulation of green and yellow pigments than did blue during the early stages. At the same time the short wave part of the spectrum increased the intensity of respiration; the photochemical activities of colloid solutions formed from green leaves grown in blue light were considerably higher than those of solutions from leaves grown in red light.

M. V. Golubtsova studied the part played by carotene in oxidation–reduction processes in plants The direct study of the compositions and conversions of pigments in plants involves many difficulties of method. Almost no work using the latest methods such as electron microscopy and sensitive precision spectrometry was reported; these might have given very valuable information on the linkages between the pigments and the leaf structures, the structures of chlorophyll–protein and other complexes, etc.

CHLOROPLASTS

The chemical properties and biochemical features of chloroplasts were discussed in the plenary paper of N. M. Sisiakan. The investigations of the last few years have shown the great significance of chloroplasts in various cellular biochemical functions related to photosynthesis and other associated metabolic processes. The chloroplast enzyme systems take part in the oxidation–reduction reactions of the cell and carry out certain synthetic functions in the formation of peptide bonds, in the conversions of nucleic acid, phospholipids and components of the tricarboxylic acid cycle.

The enzyme properties of chloroplasts formed the subject of a number of papers in the section: cyclophorase activity of growing cell structures, by R. M. Bekina,

peptide bond synthesis by I. I. Filippovich, lipoxidase activity in plastids of various types by A. M. Kobiakova and the biochemical changes in the lipids of chloroplasts by B. P. Smirnov.

The origin of the chloroplasts was dealt with in detail by L. P. Breslavets. S. S. Melik-Sarkisian ("Data on the chloroplast proteins") and M. S. Odintsova ("Ribonucleic acid of plastids and the changes it undergoes during growth of the organism") dealt with the chemical composition of chloroplasts. E. S. Mikhailova described certain enzyme processes connected with the photochemistry of chloroplast suspensions. A method evolved previously for determining dehydrogenase activity by the spectroscopic measurement of anaerobic thionine decolorization was used.

PHYSIOLOGY OF PHOTOSYNTHESIS

A. A. Nichiporovich ("The main problems of photosynthesis from the point of view of increasing its productivity) dealt with a most important economic problem, the possibility of increasing the intensity and productivity of photosynthesis in order to obtain larger crops. An analysis of the photoreactions and energy requirements, biochemistry, gaseous diffusion, etc. showed that each square metre of leaf could take up 30–40 g CO_2 per day, which corresponds with a photosynthetic production of 15–20 g of dry matter per square metre. On this basis, crops of 30–60 tons dry matter per hectare are possible.

O. V. Zalenskii ("The connexion between photosynthesis and respiration in plants") reported interesting findings on the intimate connexion between photosynthesis and respiration.

In "The differences in the oxidation–reduction systems of leaves and roots in relation to photosynthesis", by N. P. Krasinskii, it is shown that the characteristic E_H and pH figures for leaves are much lower than for roots. The cause of the downward E_H shift in leaves as compared with the roots is considered to be not the polarities of the above and below-ground organs but leaf photosynthesis, which gives various reduced compounds, primarily sugars.

A number of other sectional papers dealt with various physiological problems.

BACTERIAL PHOTOSYNTHESIS

V. N. Shaposhnikova ("Bacterial photosynthesis and the evolution of metabolic functions in micro-organisms") dealt with the evolutionary genetic connexion between various types of carbon metabolism. The most primitive metabolism is seen in photosynthesis by green and purple bacteria, using light as the main source of energy. Whereas the evolution of plant photosynthesis is based on a relative increase in the importance of reduction reactions, an increase in oxidative reactions is the prelude to autotrophism, since oxidation energy is utilized in the absence of light. This may be illustrated from CO_2 carbon assimilation by purpuriferous, hydrogen-oxidizing and sulphate-reducing bacteria. M. N. Nefelova (in the author's laboratory) established that a typical representative of the sulphur bacteria, *Chromatium vinosum*, is capable of assimilating the carbon of a number of organic acids, being on the way to "photoheterotrophism". It is possible that bacteria photoassimilate CO_2 via several organic acids which undergo stepwise photoreduction, and rearrangement of their carbon skeletons.

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I. L. Rabotnova studied the effect of redox potential and illumination on autotrophism and heterotrophism. It was shown that either autotrophism or heteortrophism predominated, depending on the intensity of illumination and aeration (rH_2).

E. N. Kondriat'eva emphasized that the development of *Rhodopseudomonas pallustris* was determined not only by the rH_2 but also by the partial pressure of oxygen in the medium. I. V. Maksimova studied the part of carotenoids in the assimilation of light energy by micro-organisms. For *Rhodopseudomonas* it was shown that light absorbed by carotenoids and by a second (green) bacteriochlorophyll absorption band, can be used for growth, although less effectively than infra-red where the fundamental absorption band of the pigment is situated. The substances taken from the media and used in photosynthesis do not depend on the wavelength of the light. The quantity of pigments formed, however, is determined by the spectral composition of the light.

LIGHT CULTURE

In the "Light culture of plants" section, problems connected with the use of artificial light for the raising of plants were discussed. The main contents of the sectional papers were ways and means of increasing the productivity of economic and decorative plants raised under artificial lighting conditions (B. S. Moshkov, V. M. Leman and others). Questions bearing on the theory of light culture were dealt with by S. V. Tageeva, A. B. Brandt, I. P. Pavlova and V. G. Listratova. The experiments were carried out under production conditions. A record of light conditions was kept (using a selenium photocell and a recording galvanometer). Questions of yield, some physiological and anatomical indices depending on the lighting conditions, and the contents of pigments (chlorophyll and carotenoids) were investigated.

METHODS OF ASSESSING AND INVESTIGATING PHOTOSYNTHESIS

A. P. Andreitsev, V. A. Il'ianok, P. A. Osetrov and M. V. Sokolov announced new equipment, developed by the Institute of Biophysics of the Academy of Sciences of the U.S.S.R., for assessing photosynthetically active light (300–720 mμ). One of these consists of a vacuum photocell, light filters, amplifying circuit and microammeters plus a standard light source. With this it is possible to measure illumination on planes and means spherical illumination. A modification of this instrument enables the spectral distribution of the radiation to be measured at 12 points in the range 360–700 mμ, by using interference filters placed in front of the photocell.

For measuring photosynthetically active light intensities a photometer has been designed which can work from the mains or from a battery and is independent of small supply voltage changes.

S. N. Chmora, V. P. Kornil'ev, L. N. Bell and A. A. Nichiporovich have constructed a field photometer for measuring the amount of radiation (400–720 mμ) falling on the spherical integrating surface during any given time.

Besides the papers read in this section, there were many papers dealing with isotopic tracer methods. The use of isotopic carbon, nitrogen and oxygen facilitated study of the primary products of photosynthesis and the effect of various factors on the rate of CO_2 absorption, and consequently on yield. The processes of carbon transformation and the nature of the primary and secondary products of photosynthesis were the subjects of several papers.

In E. A. Voichenko's paper it was shown that the primary products of photosynthesis are hydroxyacids $(CHOH.COOH)_n$, containing phosphorus and iron. Later these compounds are converted into carbohydrates and other products. All conversions of the primary products are associated with a change in the state of the iron in the main enzyme system.

N. C. Doman, using labelled CO_2 and enzyme poisons, obtained results which, in his view, confirm the occurrence of a stage of labile CO_2 linkage preceding the formation of phosphorylated acids.

The question of the first carbohydrates formed in photosynthesis was considered by M. V. Turkina. In plants which ultimately produce a very varied group of carbohydrates, with relatively short (5 min) exposures, most of the ^{14}C was in saccharose. When the plants were placed in an atmosphere of "unlabelled" CO_2, ^{14}C disappeared from the saccharose and accumulated in other carbohydrates.

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T. F. Andreeva studied protein synthesis in photosynthesizing leaves. Using ^{15}N she showed that even in very short exposures inorganic nitrogen is incorporated into the amino acids and proteins of leaves. Not only carbohydrates but also photosynthetic products containing nitrogen migrate from the chloroplasts into the cytoplasm, and ultimately into other organs of the plant. The author noted, however, that light does not always stimulate the synthesis of protein in leaves.

V. A. Chesnokov, A. A. Baskakova, L. S. Belozerova and N. S. Mamushina advanced another explanation for the action of light on protein synthesis: light stimulates carbohydrate synthesis and this provides the conditions for protein synthesis. In their experiments, injected glucose gave more powerful stimulation of protein synthesis than intense light stimulation in photosynthesis. A. M. Kuzin and G. N. Saenko showed that the $^{14}CO_2$ acceptors in the leaf are compounds of low molecular weight. Using differences in solubility and the R_f in paper chromatograms, they demonstrated seven primary "labelled" products of photosynthesis.

Isotopic tracer methods were applied with equal success in work on various factors influencing photosynthesis. N. P. Voskresenskii investigated the role of the spectral composition of light in carbohydrate conversions. During photosynthesis in plants exposed to various parts of the spectrum, considerable differences in the activities (^{14}C) of organic acids, amino acids and carbohydrates were noted. It was shown that when leaves were acted on by short-wave radiation, there was a greater consumption of oxygen than with light from the long-wave region.

E. Ia. Ermolaeva studied diurnal changes in photosynthesis in potatoes and tomatoes. It was shown that at noon, the time of the daily depression of photosynthesis, there was a fairly powerful assimilation of oxygen from the atmosphere. An increase in the concentration of CO_2 in the atmosphere led to a sharp increase in the assimilation process. The CO_2 accumulated was then used over the whole day.

A study of photoperiodism and photosynthesis in plants was carried out by Iu. I. Novitskii with an apparatus which ensured the treatment of an individual leaf of an otherwise undamaged plant and also the safety of the investigator. In the bottom of the apparatus lay transparent "Perspex" chambers through which was blown labelled CO_2, which was afterwards absorbed by alkali.

E. M. Eidel'man and A. L. Litvenenko dealt with the important question of the effect of hybridization on photosynthesis. It was shown that the seedlings of the

majority of the hybrid forms of maize display a higher intensity of $^{14}CO_2$ assimilation and a greater intensity of migration of assimilation products in the direction of the developing leaves and root system than in seedlings of inbred varieties.

The employment of labelled CO_2, as this Conference has shown, has proved very fruitful not only for the study of photosynthesis in the higher plants and green algae, but also for investigation of red algae. Adding labelled carbonate to sea water, S. I. Lebedev studied the distribution of activity in spots on the chromatogram which represented various pigments. It was demonstrated that the greatest activity was concentrated in the spot at the origin, less in the spot of yellow pigment and least in that of green pigments.

A study of the isotope composition of the oxygen from algal cells during photosynthesis was carried out by M. V. Ulubekova, ^{18}O and ^{14}C being employed. Her findings demonstrate the existence of a single mechanism for the reduction of CO_2 under aerobic and anaerobic conditions.

The extremely important practical problem of the productivity of photosynthesis in the phytoplankton of reservoirs may also (as Iu. I. Sorokin demonstrated) be decided by using labelled CO_2.

With every day the problem of photosynthesis attracts an ever wider circle of investigators. The Second All-Union Conference on Photosynthesis has recorded the successes attained and has indicated the directions for the further development of this problem, one of immense scientific and practical importance.

Iu. A. Vladimirov
S. V. Konev
F. F. Litvin

Translated by R. Crawford

SESSION OF THE COMMISSION ON PHYSIOLOGICAL OPTICS*

One of the regular sessions of the Commission on Physiological Optics was held on 25 October 1956 at the Pavlov Institute of Physiology under the chairmanship of V. V. Sharonov, Professor at Leningrad University. Papers by O. D. Barteneva and N. T. Fedorov were read and discussed.

The first dealt with the accuracy of estimations of visual brightness contrast using a special photometer (photometer-contrastometer). The author, O. D. Barteneva, stated that, if the known errors in contrast measurements with an apparatus are allowed for, the accuracy with which meteorological visibility distances (atmospheric transparences) are measured using the contrast between remote objects can be computed.

It was shown that the true contrast of an object against its background varies within certain limits. Instrumental measurements of visibility were comparable in accuracy with the simplest visual assessments.

* *Biofizika*, **2**, No. 3, 399 [Reprint Order No. BIO 51].

In the discussion Professor V. V. Sharonov stated that instruments for determining atmospheric transparency (smoke-meter, visual photometer) were of low accuracy, as Barteneva's data showed. Since these instruments were not in general use, the matter was of small importance. At the same time these instruments might have been of value in determining certain parameters required for calculating visibilities.

Professor P. M. Tikhodeev remarked that the angular separation of the fields and the relative brightness of the intervening field must be allowed for in such work. The importance of Barteneva's work was that it demonstrated that the adequately trained eye could assess contrast.

Professor N. G. Boldyrev pointed out that an interesting correlation between successive readings in the work could be observed, and that the author's findings should be elaborated and published.

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Professor N. T. Fedorov, in his paper on colour contrast, summarized data obtained by himself and his co-workers on simultaneous and successive colour contrasts for the spectral colours; he also analysed the data of Bongardt and Smirnov. He demonstrated their experimental apparatus and reproduced all their results on it, at the same time indicating that they are mainly consequences of the laws he discovered (as, for example, the change in contrast colour produced by introducing red into a saturated yellow field while changing the brightness of the latter) together with the well-known hue changes accompanying brightness reductions, and also the appreciable scattering of the bright light from the inducing background in the eye, which had already been described by Helmholtz.

In the discussion N. I. Pinegin noted that the data of N. T. Fedorov and his co-workers in the field of colour contrast are the most accurate and reliable available in the world literature. Also the results of Bongardt and Smirnov (the numerical values, he suggested, should be made available) do not in any degree refute, but merely confirm these arguments. It would have been useful if Bongardt and Smirnov had been personally acquainted with Professor Fedorov's equipment and with his experiments.

Professor V. V. Sharonov remarked on the value of Fedorov's work and pointed out that observations of certain objects in astronomy are affected by colour contrasts. The green regions on Mars may result from contrast.

Professor P. M. Tikhodeev remarked in connexion with experiments of Bongardt and Smirnov that it was very important to bear in mind the purity of the method. The use of filters was dangerous, and a monochromator should be employed.

Professor P. O. Makarov also commented on the great interest of Fedorov's work on colour induction, and drew attention to the possibility of investigating the mechanism of colour induction from the point of view of the perielectron.

Professor N. T. Fedorov, in a final word, agreed with Professor Makarov's remarks on the perielectron, and stated that he had already indicated that the colour contrast phenomena could be explained from this point of view in his combined paper, published in *Priroda* in December 1954.

V. V. SHARONOV

Translated by R. CRAWFORD

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INTRACELLULAR RECORDING OF POTENTIALS OF MUSCLE FIBRES: SINGLE STIMULI*

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(*Received* 14 *January* 1957)

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THE recently developed method of intracellular recording of electrical potentials has opened up great possibilities for detailed study of excitation processes in various muscle and nerve cells. By using this method the activity of single cells can be studied without separating them out, in conditions in which their natural connexions with their neighbours are preserved. In muscle fibres, using intracellular microelectrodes, one can solve such problems as the spread of waves of excitation in any fibre, the physico-chemical properties of their membranes, the mechanisms of direct and indirect stimulation, etc. Many of these questions, until now, either have been little studied or are the subject of quite heated discussions.

We have carried out an investigation of the activity of striated muscle fibres using this method. In the present communication we report part of the results obtained, on the electrical potentials of inactive fibres and also with single direct and indirect stimuli. Subsequently we shall report results obtained with repeated and rhythmic stimulation. The ultra-microelectrode technique has already been applied to muscle fibres by a number of investigators [1–8]. Especially great attention has been paid to the study of membrane potentials of muscle fibres and to the responses to single stimuli. The processes which set in on repeated and rhythmic stimulation have been studied considerably less. However, we considered it necessary, before going over to tackle the more complex questions connected with rhythmic activity of muscles, once again to stop at the simpler phenomena connected with single stimuli. A preliminary communication on this work has been published earlier [9].

METHODS

Our intracellular microelectrodes were glass micropipettes filled with 3 M KCl. The pipettes were drawn out by a powerful electromagnet from small electrically heated hard glass tubes. The strength of the magnet was changed in proportion to the heating, as was proposed by Alexander and Nastuk [10]. At first the magnet strength was small, but when the capillary was somewhat drawn out, it was abruptly increased (10 times). The switch-over was carried out by a relay system when the capillary had reached a determined length; simultaneously the heating was automatically switched off. Thanks to this process the tip of the capillary took a conical shape; the tip did not seal off and the external diameter was a few tenths of a micron. By adjusting the strength of the magnet, the kind of glass and the degree of heating, it was possible to obtain microelectrodes with very sharp points, which is important for minimizing the injury to the fibres when they are pierced.

*Biofizika **2**: No. 4, 401–416, 1957 [Reprint Order No. BIO 51].

The filling of the microelectrodes was carried out using a preliminary boiling in methyl alcohol at room temperature in a vacuum. Then the electrodes were placed for 24 hr in KCl, when the alcohol was replaced (any other electrolyte could be used). Such a method is more advantageous than direct boiling, which has been used by many authors. The low temperature and even boiling due to the change in pressure, considerably decrease the percentage of broken tips. The electrodes were placed then and there, tips down, in great numbers, in a special Plexiglas holder in which they were transferred from the first solution to the KCl solution in which they were stored after filling. As a rule the electrodes were used within a few days of being filled; if stored longer, crystallization often occurred in the capillary and the electrodes became unfit for use.

The use of a concentrated electrolyte solution as a filling is necessary so as to decrease the microelectrode's resistance, which is as much as 10–20 MΩ even at 3 M concentration. The use of KCl decreases the diffusion potential which can arise between the filling and the cell protoplasm. Indeed, even with a KCl filling, the microelectrodes have some potential when in contact with another solution. When dipping into Ringer's solution such a potential, for various microelectrodes, ranges between 0 and 30 mV, its mean equalling 9·3 mV (the potential of the microelectrode being always negative with respect to the solution). The magnitude of this potential depends on many factors, e.g. the ratio of the solution concentrations inside and outside the microelectrode (the potential decreases on equalizing the concentrations, although it does not quite disappear) and the size of the hole in the microelectrode (breaking the tip decreases this potential). The circumstance that an electrode potential (mean of −6·0 mV) exists even when the concentrations inside and outside are the same, suggests its partial dependence on electrical phenomena being developed in the glass. This part of the potential can apparently vary according to the kind of glass used.

If the potential for each electrode is constant, then it need not appear in the experimental results (under the condition in which the electrode is not taken out of the liquid in which the investigated muscle is placed). However, such a potential can change during the course of an experiment. This change can be caused by breaking or plugging the tip when it penetrates into the cell [8]. Such damage to the electrode can usually be detected under the microscope. Otherwise a change in the microelectrode potential can take place because of a difference between the ionic composition of the protoplasm and the ionic composition of the Ringer's solution surrounding the fibre. We cannot directly measure the magnitude of the potential arising between the filling of the microelectrode and the cell protoplasm. Comparison of the magnitudes of the potentials which arise when the same microelectrode is dipped into Ringer's solution and into a solution containing the ions K, Na and Cl in the same proportions as in muscle fibre protoplasm (15 mg equiv. Na, 125 mg equiv. K and 1·2 mg equiv. Cl, the rest of the anions being replaced by HPO_4), shows that, in the second case, the negative potential of the microelectrode is, on the average, 5·8 mV greater. Therefore one ought to consider that this change of electrode potential is added to the true value of the membrane potential on penetration of the electrode through the surface of the cell. In stating our results we have not carried out this correction since it is impossible to calculate it accurately (in view of the absence of exact data on the ionic activities in the protoplasm).

Filling the microelectrode with 3 M KCl possesses this drawback, that electrolyte diffusion into the cell can take place. However, with a complete tip to the microelectrode, such diffusion is so insignificant that it cannot give rise to noticeable disturbances of the vital activity of the process under investigation. Thus, according to the calculation of Nastuk and Hodgkin [2], with a tip diameter of a few tenths of a micron the speed of diffusion amounts, in all, to 6×10^{-14} moles/sec.

The microelectrode was connected by means of rubber tubing, also filled with KCl solution, with a non-polarizable leading-off electrode (a chlorided silver wire in KCl agar). A second such electrode (reference) was put directly into the solution surrounding the muscle. Using non-polarizable electrodes filled with NaCl agar increases the electrode potential. The microelectrode was connected to the grid of the input valve and the indifferent electrode was connected through a calibrator to earth. The input valve, working as a cathode follower, was directly mounted on a micromanipulator by means of which the microelectrode was introduced into the fibre. We have described this kind of scheme previously in detail [11]. It allows one to use high-resistance microelectrodes for recording the most rapid bioelectric fluctuations. Since the over-all input capacity totalled 2·5 pF, the time constant when using a microelectrode of maximum resistance (17–18 MΩ) was of the order of 40 μsec. An important condition for the success of intracellular recording is a minimum input valve grid current. In our arrangement it did not exceed 10^{-11} A. Such a grid current value could not give rise to noticeable polarization changes of the surface of the muscle fibre. Since the grid current might change (because of small impurities in the glass, etc.), its value was checked during the experiment by comparing the position of the oscillograph beam when the input grid was connected directly to ground and through a resistance of 50–100 MΩ. Displacement of the beam proved the presence of grid current which caused a potential drop across the shunting resistance; in such a case the grid current was reduced to a minimum by adjusting the anode potential of the input valve. In the circuit of the indifferent leading-off electrode there was permanently connected a low-resistance calibrator by means of which, during an experimental run, the amplification of the apparatus (amplifier) and the resistance of the microelectrode could be calibrated. The latter was measured by comparing the deflexion of the oscillograph beam when the same constant signal was delivered by the calibrator with and without a 50 MΩ shunt across the microelectrode. By constantly checking the resistance of the microelectrode during an experiment, we can tell whether the tip of the electrode breaks or gets plugged up. We have described earlier [11], in more detail, the input circuit and the method of measuring the resistance of the microelectrode. The input valve was connected to a d.c. two-valve amplifier; the amplifier output was connected to the vertical deflexion plates of a cathode-ray oscillograph tube.

The experiments were carried out on the sartorious muscle of the frog. The muscle was fixed, isometrically, in a special Plexiglas chamber which allowed illumination of the preparation from any side; introduction of the microelectrode was checked with the help of a powerful binocular magnifier. With this, only the direction of the microelectrode towards the desired fibre was established. The instant of piercing with the whole tip of the microelectrode is usually not observed, since it is not accompanied by a depression of the fibre surface; it is only detected, by means of

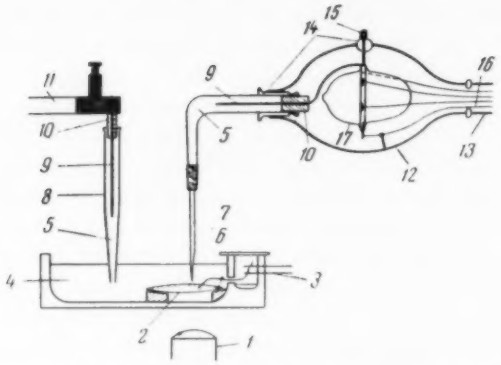

FIG. 1. Arrangement for intracellular recording of the potentials of frog muscle.
1—illumination; 2—muscle; 3—stimulating electrodes; 4—Ringer's solution; 5—KCl-agar; 6—KCl; 7—microelectrode; 8—indifferent electrode; 9—Ag–AgCl; 10—plug; 11—connexion of electrode with calibrator; 12—valve screen; 13—earth; 14—insulator; 15—terminal of shunting resistance; 16—connexion to power supply; 17—input valve.

the oscillograph, by a jump in the membrane potential. A significant depression of the cell surface always means a broken electrode tip. As a standard, Ringer's solution was taken, similar to that recommended by Nastuk and Hodgkin, namely: 116·25 mg equiv. Na; 2·5 mg equiv. K; 3·6 mg equiv. Ca; 117·1 mg equiv. Cl; 0·75 mg equiv. H_2PO_4; 4·5 mg equiv. HPO_4 per l. Stimulation was by single induction pulses; for direct stimulation, two needle-shaped electrodes, insulated along their length except at the very tip, were introduced into the muscle. The experiments took place at room temperature (18–20°).

Fig. 1 shows the general arrangement for intracellular recording of the potentials of the sartorius muscle of the frog.

RESULTS

Potentials in the absence of stimulation

If the microelectrode diameter was less than 1μ and penetrated the muscle fibre without noticeable depression of its surface, then at the instant of penetration the occurrence of a jump in the d.c. potential difference was always recorded. At the same time the microelectrode became negative with respect to the reference electrode. Further electrode penetration into the protoplasm was not accompanied by changes in this potential difference. After passing through the opposite surface of the fibre the potential difference vanished. Thus this difference of potential is connected with the fibre surface and we shall subsequently call it, conventionally, the membrane potential (M.P.). According to measurements, carried out in our laboratory by Z. A. Sorokina, on 300 fibres, it averages 83 mV (ranging from 73 to 117 mV). If the muscle remains in Ringer's solution, of the above-indicated composition, then the M.P. gradually decreases (in absolute magnitude). According to Sorokina's data, it decreases during the second half-hour to 82·5 mV (range 65–114 mV), during the third to 81 mV (range 60–106 mV) and during the fourth to 80·4 mV (range 60–105 mV). We have detected no dependence of the magnitude of the M.P. on the diameter of the muscle fibre.

If the penetration of the fibre was carried out with a microelectrode of tip diameter greater than 1μ then one could observe not a sharp jump, but a gradual appearance of the M.P. Its growth coincided with the depression of the fibre surface due to the microelectrode pressure and was probably the result of this depression. The final value of the M.P. after penetrating into the cell in such cases, often seemed considerably smaller than the value usually obtained. Such results were disregarded in determining the natural value of the M.P.; they suggest that mechanical pressure can depolarize to some extent the surface of the fibre.

Action potentials

With both direct and indirect single stimulation of muscle, and intracellular recording, action potentials (A.P.) were detected in many fibres; their magnitude during the first hr after preparing the muscle reached +126 mV above the level of the M.P. (the mean value obtained on 35 fibres was +105 mV). Thus the surface of the fibre, while the action potential was developing, acquired a potential difference opposite in sign to that of the inactive state. The excess of A.P. over M.P. (overshoot) averaged 22 mV. The A.P. was always monophasic and its length (without the "after" negative part) was about 3·5 msec. A typical example of an A.P. is given in oscillogram* 2 (Fig. 2a). An A.P. was not recorded in each muscle fibre examined; in some

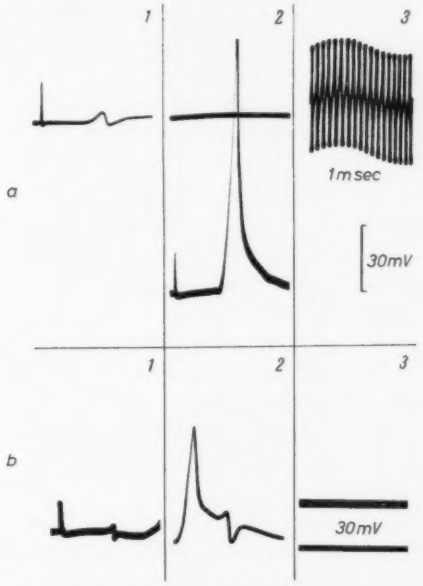

FIG. 2. (a) Curarized muscle, direct stimulation. Oscillograms: 1—potential on locating the microelectrode close to the surface of an excited fibre; 2—after penetration into the same fibre. (b) Indirect stimulation, recording in the middle of the muscle. Oscillograms: 1—microelectrode in an unexcited fibre polarized by the action current of a neighbouring fibre; 2—microelectrode in an excited fibre, the same polarization; 3—scales of time and magnitude of potential.

*In all the oscillograms, in distinction from the designation used when recording is done by an extracellular method, an upward deflexion of the beam corresponds to the active electrode being electrically positive.

fibres no changes in M.P. on stimulation were noted. This evidently signifies that, with both direct and indirect stimulation, not all the muscle fibres were excited. Also this proves without doubt that the intracellularly recorded A.P. is connected with the excitation of just that fibre into which the microelectrode is introduced, and is not a consequence of the drawing-off of an action current of the whole muscle.

If the microelectrode is withdrawn from a fibre, in which an A.P. has been recorded, and placed in direct proximity to the fibre surface, then it is possible to record a small (up to 5 mV) tri- or di-phasic potential, which appears therefore to be the result of extracellular recording of the bioelectric potential of the excitation process. The multiphasicity of such spikes is due to the method of recording biopotentials in a volume conductor by means of a point electrode. In such recording, the microelectrode does not directly reproduce the change in M.P., but registers in relation to the fibre the potential drop in the external medium, which is created by action current loops going from the positively charged inactive surface of the cell to the negatively charged active part. If at the same time the second, reference, electrode is far from the cell and records a practically unchanged zero potential, then the microelectrode records, with respect to this remote electrode, a positive potential difference when it is located in front of or behind the active region where the action current leaves the cell. When the wave of activity appears directly under the microelectrode then a potential is recorded by the microelectrode, which is negative with respect to the distant electrode.

In Fig. 2(a) (oscillogram 1) a potential spike is shown which was recorded extracellularly, near the surface of the excited fibre in which was recorded the A.P. shown in oscillogram 2 (Fig. 2a). The difference between the base lines of oscillograms 1 and 2 corresponds to the magnitude of the M.P. of the fibre. Of course such extracellular recording of action currents occurs not only with a microelectrode position close to the surface of the muscle, but also when it is placed inside some fibre—if at the same time a neighbouring muscle fibre is excited. The current loops, which arise on such excitation in the medium surrounding the fibre, in part enclose neighbouring fibres too. In their nature the potential spikes which are recorded in this case do not differ in any way from those recorded extracellularly, directly near the cell surface. We succeeded in detecting these spikes both when the investigated fibre remained itself unexcited (oscillogram 1, Fig. 2b), and when the investigated fibre was excited. If the fibre which had a microelectrode in it was excited, then the effect from neighbouring fibres was observed on some part of the A.P. of this fibre. It is important to note that when the spikes, evoked by a neighbouring fibre, coincide with the A.P., they appear considerably amplified, a circumstance to which Easton [7] has also paid attention. Evidently when a fibre is excited its membrane resistance is considerably decreased and therefore the strength of the action currents of the excited fibres, penetrating into the fibre from which the potential is being recorded, correspondingly increases (oscillogram 2, Fig. 2b). Often from some fibres one can record not one but two or three such small di- or tri-phasic spikes which clearly are due to the influence of a few excited neighbouring fibres. These spikes do not coincide because of the unequal distance, in different fibres, between the position of innervation and the position of recording.

If, in spite of the fibre contraction which always accompanies an A.P., the microelectrode in the fibre remained whole, then it was possible for a short time to record an unchanged A.P. With both direct and indirect stimulation (and recording at a distance of 1–2 cm from the place of stimulation) and with a gradual increase in stimulus from a subthreshold value, gradation of the A.P.s was not observed if the magnitude of the M.P. remained unchanged (Fig. 3a). Occasionally, inconsiderable

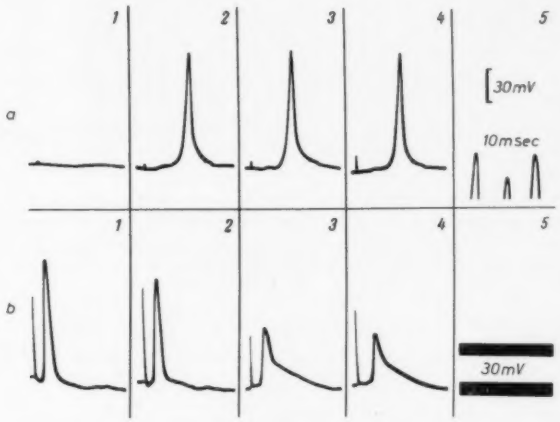

FIG. 3. (a) Indirect stimulation, recording from a fibre in the pelvic part of the muscle: Gradually increasing stimulus. (b) Direct stimulation, recording from a fibre at a distance of 1 cm from the stimulated spot. Change of the action potentials in the case of injury of the fibre by the microelectrode; oscillograms taken with a constant strength of stimulus at intervals of 1 min.

fluctuations in the magnitude of a series of successive A.P.s, recorded from one and the same spot of a fibre, took place; those fluctuations did not depend on changes in intensity of stimulus and did not exceed a few millivolts. This does not indicate that the spike of the propagated pulse has the same intensity over the whole extent of the muscle fibre. To trace an A.P. over a considerable length of one and the same fibre is scarcely possible, since the fibres overlap each other. However, if we compare the magnitudes of the A.P.s in the myoneural and nerve-free regions of a large number of fibres, then the myoneural A.P.s are usually 10–15 mV smaller than the nerve-free (Fig. 4). The mean value of the A.P. in a nerve-free region was +111 mV, in the myoneural region it equalled +103 mV. It is possible to look at this as evidence of the weakening of the spike near the motor end-plate (the region of the muscle fibre directly adjacent to the nerve ending) in connexion with the shunting action of the lengthy depolarization of the latter [3]. But, considering the above-mentioned increase in the passage of the external electric currents into the fibre during its excitation, one can quite probably put forward the hypothesis that the weakening of the A.P. in this region is an artifact, due to the superposition of external electrical fields (of the pre-synaptic endings and neighbouring muscle fibres) on the intracellular A.P.

It was quite different when the microelectrode was too large and injured the muscle fibre: the value of the A.P. rapidly decreased with time, in distinction from

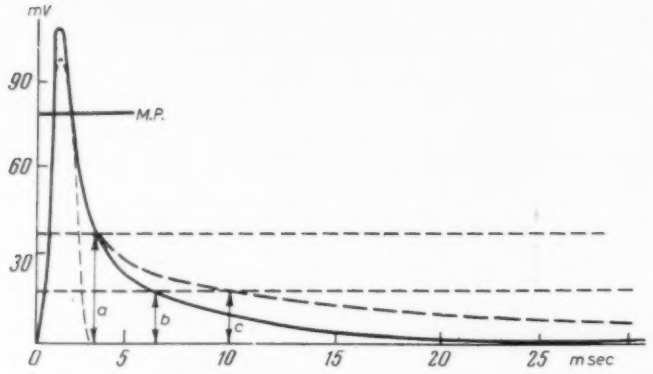

FIG. 4. Comparison of action and after potentials, recorded from a fibre in the region of the myoneural junction (broken line) and in the nerve-free region (unbroken line). M.P.—membrane potential; (a) maximum size of the negative after potential; (b) its half-intensity in the nerve-free region; (c) the same in the region of the myoneural junction.

the M.P. which appeared much more stable to the injuring action of the microelectrode. After a time the A.P. became numerically equal to M.P. and later still became less than it. At the same time, as a rule, the descending phase of the potential was drawn out and developed a quite lengthy "after" electro-negativity. At the very end the spike began to suggest a local potential. Thus regular A.P.s are intrinsic only to completely undamaged fibres. On altering the muscle fibres the conduction of the waves of excitation quickly changes. Decreasing spikes arise which are then converted into local processes.

Oscillograms are shown in Fig. 3b illustrating the changes in the A.P. of a muscle fibre pierced by too large a microelectrode.

By measurement of the latent periods of A.P.s on direct stimulation of a curarized muscle, we have determined the speed of conduction of excitation in various muscle fibres. In order to exclude the influence on the results of the magnitude of the effective time of stimulation of different fibres, we determined the conduction speed by the difference of the latent periods of the A.P.s recorded at one and the same spot on the fibre, but on decreasing the distance from the place of stimulation by a factor of two. In such cases the latent period was also shortened by a factor of two; consequently the difference in effective time of stimulation of different fibres did not play a substantial role in the determination of the latent period of the A.P.s In most fibres the conduction speed varied from 2·5 to 4 m/sec. However, sometimes fibres were encountered with a much smaller conduction speed—from 0·8 to 1·5 m/sec. Proportionality between the magnitudes of M.P. and A.P. in different fibres was not observed. But if in some fibre the M.P. for some reason or other decreased, then the A.P. also weakened proportionally.

After potentials

A characteristic feature of the electrical potentials of propagated impulses in muscle fibres is the existence of the so-called after potentials (S.P.) which continue after the disappearance of the action potentials (A.P.). As a rule the A.P. is accompanied

by a well-expressed negative S.P., without any noticeable subsequent positive S.P.* The magnitude and length of the S.P. were quite different in different parts of a muscle fibre; this was shown some time ago by Serkov [12], and more recently by Vorontsov [13] and Nastuk [6]. On recording potentials from the myoneural region, the S.P. was especially pronounced and long (oscillogram 2 in Fig. 6b). And the magnitude of the S.P. quite quickly decreased according as the microelectrode was implanted still further from the end plate (compare oscillograms 2 and 6 in Fig. 6). In Fig. 4 both types of S.P. are superposed and the differences between them appear especially well. It can be seen that the magnitude of the negative S.P. reaches 35 mV (a). The time to fall to half (considered from the time of disappearance of the A.P.) in the nerve-free region is about 3·5 msec (b), but in the myoneural region is about 8 msec (c).

The over-all length of the S.P. in the myoneural region is about 20 msec, and in the nerve-free is 40–60 msec.

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In some cases we observed the passing-over of an A.P., not into a negative, but into a positive S.P. We did not succeed in establishing the reasons for the occurrence in a fibre of such a rapidly developing hyperpolarization. It is only possible to say that the positive S.P. is very variable and quickly disappeared on repeated stimulation, giving way to the usual negative S.P. In a few fibres we observed also the opposite phenomenon, during long recording of potentials from one and the same fibre, in which the A.P. gradually decreased, there was observed not the usual increase and lengthening of the negative S.P., but its transformation into a distinct, though small, positive S.P. (Fig. 5a). The magnitude of the positive S.P.s which we observed, did not exceed 10 mV. In Fig. 5b oscillograms of positive after-potentials are shown.

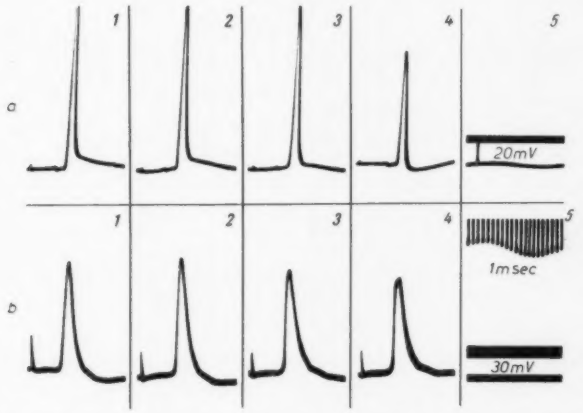

FIG. 5. (a) Indirect stimulation, recording from a fibre in the middle of the muscle. Transformation of a negative after-potential into a positive; oscillograms taken with stimuli of constant strength at intervals of 1 min. (b) Curarized muscle, direct stimulation. Positive after-potential replaced by negative. Oscillograms taken at the same intervals.

* By the terms "negative" and "positive" we understand, respectively, depolarization and hyperpolarization of the membrane (although the changes of potential, recorded by the intracellular electrode, were in fact in the opposite direction).

Local potentials

The most distinct local potentials (L.P.) can be recorded in the region of the end plate, especially when the myoneural transmission is blocked by curare or fatigue. The corresponding parts of the muscle fibres can be found under the microscope when the branches of the motor nerve are vitally stained (e.g. with methylene blue). This often disturbs the conduction of excitation in the presynaptic fibres, but not in the synaptic connexions; and then the L.P. is not recorded. It is more convenient to investigate a series of muscle fibres in the region of their innervation without previous staining. If a muscle is curarized and does not contract, then with one and the same microelectrode—without breaking its tip—one can pierce a large number of fibres and quickly find one in which a L.P. is recorded. By implanting the microelectrode into different spots of such a fibre, one can find that part in which the L.P. is maximally expressed and, clearly, where the nerve–muscle junction is situated.

The typical L.P. of such a part, obtained with the nerve–muscle transmission fully-blocked by curare, is shown on the oscillograms in Fig. 6a. This potential quickly grew to a maximum and then exponentially fell to zero. The size of the L.P.

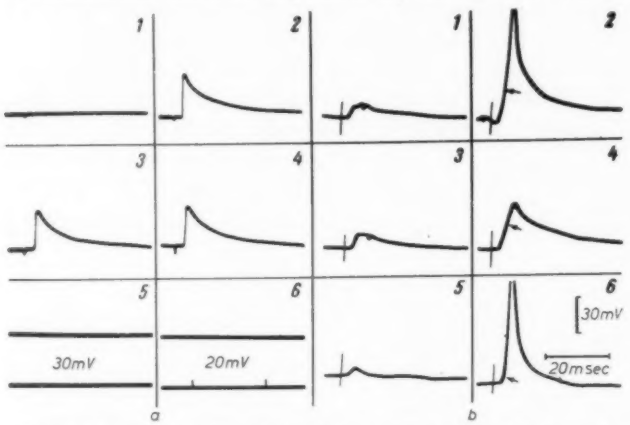

FIG. 6. (a) Curarized muscle, indirect stimulation. Local potentials from the end-plate region with gradually increasing stimulation of the nerve. (b) Potentials from the same region of the muscle fibre after a longer time in the Ringer's solution. Oscillograms: 1 and 3—local potentials; 2—local potential converted into a spike on its summation with a preceding pulse; 4—"abortive" spike when less summation of the local potentials occurs (a longer interval after the previous nerve impulse); 5—local potential on placing the microelectrode at a greater distance from the nerve ending; 6—conversion of a local potential into a spike.

in a curarized muscle reached 25–30 mV and the time for fall to half was 4–5 msec. According to how much the microelectrode was moved to one or other side from the myoneural junction, so the L.P. decreased. It also changed its shape: the rising phase became less steep, the falling more sloping. The L.P. completely vanished at a distance of about 3 mm from the place where it was maximally expressed.

According to all these signs, the described L.P. corresponds to those potentials detected in the end-plate region by other investigators [13–17], which have received the name "end plate potential" (e.p.p.). The A.P. is the change in M.P. of the muscle fibre, and not the potential of the nerve ending, since it vanishes simultaneously

with the disappearance of the M.P. when the microelectrode is withdrawn from the fibre. In this latter case only a very weak spike in the opposite direction is observed on stimulating the nerve.

The magnitude of the L.P. did not change when the strength of stimulation of the nerve was changed (Fig. 6a), and its "gradation" could be evoked only by the action of curare or other blocking agents—and also by the interaction of subsequent L.P.s. A characteristic feature of the end-plate L.P.s is their stability to damaging influences. Such potentials can be recorded without change from one and the same fibre over tens of minutes. Even when the fibre was damaged by a large electrode, quite usual L.P.s were recorded which, unlike the A.P.s, did not decrease with time.

If the blocking effect was hardly sufficient to cause cessation of nerve–muscle transmission, then on increasing the L.P.s by summation they could be converted into a propagated spike. In such cases the A.P. appeared when the rising part of the L.P. attained a definite critical magnitude. The instant of transfer of an L.P. into an A.P. could be detected by a small kink in the rising slope—the A.P. has a steeper slope than the L.P.

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The transformation of an L.P. into a spike is shown on the right oscillograms of Fig. 6b. Oscillogram 1 shows an L.P. recorded from the myoneural part of a muscle fibre in response to a single stimulation of the motor nerve. Block of the nerve–muscle transmission arose in this fibre not with curare but as a consequence of fatigue. The slower rise and fall of the L.P. in this case is explained, in the first place, by recording at some distance from the nerve ending and, in the second place, by the absence of curarization which leads to a quicker vanishing of the local potential. On the background of the L.P. is seen the effect from an excited neighbouring fibre. Oscillogram 2 shows the same stimulus applied on the background of an L.P., summating with the previous one, and transforming it into an A.P. (the probable place of transformation being marked by an arrow). In oscillograms 5 and 6 analogous phenomena are shown but with implantation of the microelectrode at a still greater distance from the nerve ending; the L.P. was more weakly recorded, the A.P. was accompanied by a much smaller negative S.P., which is characteristic for the nerve-free region of a muscle fibre (see above).

The critical level at which the local process transforms into a propagating pulse was from 30 to 40 mV.

It should be noted that in some cases we observed the appearance on the oscillogram of L.P.s not maximal, but submaximal, "graded" spikes (oscillogram 4 in Fig. 5).

Such "abortive" A.P.s were observed when the L.P. was only slightly increased by the preceding impulse, so that it scarcely reached the critical level. It is probable that "abortive spikes" arise in muscle fibres comparatively rarely, though in some cases as, for example, in invertebrate nerve cells, they are regularly encountered [18].

In some fibres, in response to stimulation of the nerve, it was possible to detect L.P.s which were distinguished from those described above by very great length. They could also be recorded without blocking nerve–muscle transmission. This definitely suggests that such L.P.s cannot be accompanied, in normal conditions, by A.P.s; in most fibres the L.P., evoked by a motor impulse, is always above-threshold in respect of the propagating impulse. It is possible that such potentials are analogous to the slow fluctuations of potential described by Kuffler and Williams [5] and

Zhukov [19] and characteristic, according to their data, of tonic muscle fibres, which are innervated by thin fibres in the body of the motor nerve.

Of course it is of great interest to consider whether the natural development of the L.P. is only in the end-plate region or whether it is also characteristic of the whole muscle fibre. Our data incline us towards the second suggestion. We have observed, on direct stimulation of a curarized muscle, long "graded" L.P.s which in some cases reached the critical level and generated typical spikes. Very often the reason for the arising of such L.P.s was mechanical stimulation of the fibre by the microelectrode during muscle contraction, which we shall discuss a little later. A characteristic example of such an L.P., reaching the critical level and evoking an A.P., is oscillogram 5 in Fig. 7. From the long latent period of the potential change it is evident

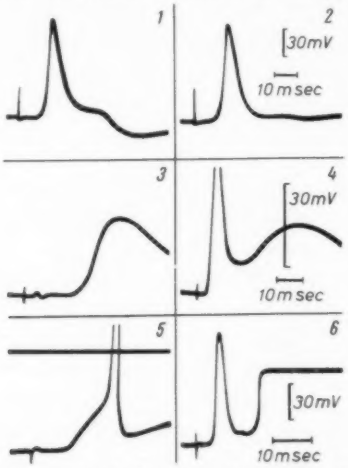

FIG. 7. Upper series—indirect stimulation, recording from a fibre in the pelvic region of the muscle; hyperpolarization at the time of contraction. Middle series—indirect stimulation; partial depolarization at the time of contraction. Lower series—direct stimulation. Oscillograms: 5—local depolarization of the fibre, generating a spike; 6—disappearance of the membrane potential difference when the microelectrode jumps out of the fibre.

that it was not directly caused by the electrical stimulus; the L.P. began at the same time as the muscle contraction. Since in itself the muscle contraction is not accompanied by any electrical effects (see below), it is clear that the changes in M.P. were caused by the stimulating action of the microelectrode at the time of contraction. The possibility is not excluded that in some cases (the microelectrode breaking) the stimulating factor is just a flow of KCl out of the microelectrode.

The course of the L.P. in the nerve-free part of the muscle fibre differs from that in the region of the end plate by its very slow rise and fall. However, the critical values of the change of M.P. necessary for the generation of a propagated spike were the same in both cases and did not differ from the critical value when generating a spike by catelectrotonus, produced by an external electric current.

The L.P. of the myoneural region was well registered in a muscle which had been strongly poisoned by curare and whose fibres did not contract at all. Thus the L.P. of the myoneural region, unlike the A.P., is not connected with the development of the subsequent contraction of the fibre. The question whether the L.P.s recorded

in other parts of the muscle fibre can be accompanied by contraction, demands special investigation.

The change of the potentials on contraction

In the overwhelming majority of cases, on putting a microelectrode into a fibre which contracts and gives A.P.s, no special changes in the M.P.s, which should coincide in time with the development of contraction, are observed. As Serkov[12] showed, in this period only the gradual weakening of the negative S.P., which has no direct connexion with the contraction, takes place. But in some cases it was possible to note, at the instant of contraction, some additional potential changes recorded by the intracellular microelectrode. Such changes could be of different forms. In a number of cases, after the A.P. (10–20 msec after the stimulus had been applied) there took place a sharp disappearance of the usual M.P. (see oscillogram 6 in Fig. 7). Possibly, in such a case, the microelectrode jumped out of the fibre when it contracted.

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Sometimes the complete disappearance of the M.P. at the same time as the contraction was not observed, but only a temporary, partial, membrane depolarization about which we spoke in a previous section (oscillograms 3–5 in Fig. 7). One cannot look at this partial depolarization as some kind of special S.P., since in a number of cases it arose without a preceding spike. Thus oscillograms 3 and 4 of Fig. 7 are obtained by recording from one and the same fibre but with an increase in strength of stimulation of the nerve. With the weaker stimulation (oscillogram 3) there was no A.P. in the fibre, but the partial depolarization was well expressed. With a stronger stimulation (oscillogram 4) a typical A.P. arose, on the negative S.P. of which was superposed the partial depolarization. It is clear that when the weaker stimulus was applied neighbouring fibres were excited and contracted (small potential changes, evoked by their action currents, are visible on the curve between the stimulating current and the partial depolarization). The mechanical effect of their contractions caused a movement of the microelectrode in the recording fibre which led to changes in the membrane polarization.

The gradual growth of the partial depolarization is at the same time connected, most probably, with the change in M.P. of that part of the fibre under mechanical excitation. It is not very likely that such a gradual development of electrical changes would be connected with the existence of a potential gradient in the surface layers of the fibre or with the gradual movement of the microelectrode into different parts of this gradient. For on penetration of the cell by a sufficiently thin microelectrode not denting the surface, a gradually arising potential difference is not observed even with a very slow penetration; the thickness of the layer dividing the two potentials is so insignificant that it is practically always pierced by the microelectrode at one stroke.

Finally, in some cases, during the period corresponding to the development of the fibre contraction, some hyperpolarization of the membrane could be observed. An example is oscillogram 1, Fig. 7. We did not often observe such hyperpolarization. Usually it was irregular and disappeared after a few repeated stimuli (see oscillogram 2 of the same figure). The arising of hyperpolarization was clearly not connected with the after-processes of the A.P. since it began as a distinct independent change with a considerable latent period. It is impossible to say anything definite about the mechanism of origin of this hyperpolarization. Perhaps it is an artifact connected

with a change of the microelectrode's own potential when the latter, due to the movement of the fibre, pushes into the inner surface of the part of the sarcolemma which is opposite its place of penetration.

DISCUSSION

In the light of the stated facts two questions acquire special interest. The first is the nature of the potentials recorded by a microelectrode in the interior of a fibre. When giving the experimental data we conditionally designated the potential, recorded on penetration of the microelectrode into the fibre, as the membrane potential since it arises sharply just at the instant of piercing the fibre surface. As is well known, the hypothesis which connects such a potential exactly with the surface membrane, its permeability and the ionic ratios on both sides of it, is now very strongly confirmed [20–23]. This hypothesis sufficiently well explains the physico-chemical basis of the action potential and, in particular, why its height exceeds that of the membrane potential [24]. The data which we and other investigators have obtained, about the recording of a potential difference only after piercing the fibre surface and about the appearance, on excitation, at this surface of an oppositely directed potential difference, can scarcely be explained if the role of the above-indicated mechanism in producing the cell potentials is denied. It therefore seems to us that, in this case, the use of the term "membrane potential" is fully justified. The second question is the nature of the potentials which arise in a separate fibre on stimulation. A lively discussion on the so-called "all or none" law has now lasted for many years, but it has still not led to a unanimous opinion. As well as supporters of the universal application of this rule, there are investigators who, without any basis, see, even in individual uses of its confirmation, the appearance of a metaphysical and even an idealistic approach to the subject [25].

Intracellular recording of potentials shows that, in most striated muscle fibres, the basic type of propagated excitation consists of impulses which are not graded as a function of the intensity of stimulation. There is clearly a similar situation in the motor-nerve fibres, since the local potentials in the end-plate region do not change their magnitude when the strength of the single stimuli to the nerve is changed. It should be noted that Serkov, who investigated in detail the activity of individual muscle fibres, could not detect gradation of contractions for some of them [26]. It is probable that such a mechanism of conduction of excitation takes place in those substrates, the basic function of which is the conduction of impulses to a distance. It secures a constant high speed for the nerve signal. Of course one should not think that the propagated impulses are all perfectly similar. There are fluctuations in the muscle fibre of up to 5 mV, yet these depend not on a change of intensity of stimulation but on some internal, protoplasmic, cause. Such fluctuations can also take place during the passage of an impulse from one part of the muscle fibre to another. The mechanism of maintaining the constant size of the propagated impulses in a muscle fibre is considerably less perfect than in nerve. Even the most insignificant alteration at once disturbs it and converts ungraded spikes into graded ones. If a gradation of propagated impulses in muscle fibres, depending on intensity of stimulation, has been recorded by many authors, then this must probably be connected with injury to the fibre during preparation. Our experiments clearly show that even such

a comparatively insignificant injury as piercing a fibre with a 1μ microelectrode in one spot can lead to an abrupt weakening of the action potentials and to the appearance of gradation of their intensity. And surely the possibilities of such an injury are increased a thousandfold by separating a fibre along all its length. It is possible that in a number of cases the gradation of the effects can also be explained by the appearance of "abortive spikes", especially when nerve–muscle transmission is partially blocked by some factor. But such spikes, at least in muscle fibres, are comparatively rare. We cannot as yet say anything definite about the conditions which lead to their appearance and we cannot definitely establish the possibility of their appearance in the completely normal functioning fibre.

Another matter is the arising of excitation in the muscle fibre. At the point of stimulation the fibre responds by a local process whose intensity is changed in proportion to the stimulus intensity. Of course, if the local process arises in the region of the nerve–muscle junction, then, with a constant size of impulses in the nerve fibre, one cannot expect gradation of the local responses to single stimuli. But when repeated impulses go into the synapse, such a gradation obviously completely sets in. It is in just such a dependence of the intensity of the synaptic processes on the frequency of the nerve impulses going into the synapse that, clearly, the physiological mechanism of the adequate response of the effector organ to a change of the incoming impulse consists; in natural conditions the nature of the impulse is always a rhythmic discharge, varying with frequency.

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CONCLUSIONS

(1) By means of intracellular electrodes, with a tip diameter less than 1μ, the potentials of muscle fibres of the frog sartorius muscle were recorded. The piercing of the surface by such an electrode was accompanied, in all fibres, by the abrupt appearance of a constant potential difference—on the average 83 mV. On direct and indirect excitation an action potential was recorded in the excited fibres whose mean value was +105 mV (thus exceeding the membrane potential by +22 mV). The appearance of the spike was accompanied by contraction of the fibre. In uninjured fibres the action potentials were not graded as a function of the stimulus intensity.

(2) The speed of propagation of the action potentials average, in most fibres, 3·4 m/sec, but in some fibres it was approximately three times smaller.

(3) The action potential was accompanied by a well-expressed after-depolarization. In the nerve-free region of the muscle fibre the duration of this was about 20 msec, but in the region of the nerve–muscle junction it was 40–60 msec. In some cases the action potential was succeeded by an after-hyperpolarization, but the latter was very irregular.

(4) When the nerve–muscle transmission was blocked, the nerve impulse evoked a local decrease in membrane potential, of 25–30 mV, in the region of the end plate. The duration of the local depolarization was about 20 msec. If it reached 30–40 mV a propagated spike appeared (maximal as a rule, but in some cases it was weak or "abortive"). In itself the local depolarization was not accompanied by contraction of the muscle fibre.

(5) Local depolarization processes could also be detected in other parts of the

muscle fibre where they were evoked by mechanical stimulation (by the microelectrode) at the time of contraction. Such a local depolarization differed from the local depolarization in the region of the nerve–muscle junction by its slower course. However, the critical magnitude of the depolarization necessary for the local change to go over into a propagated process was the same in both cases.

(6) The contraction of the fibre was not accompanied by any special changes in the membrane potential. The appearance in some cases of electrical potential fluctuations in this period was connected with the mechanical action of the microelectrode on the fibre during contraction of the latter.

(7) An intracellular microelectrode was able to record the action potentials of neighbouring excited fibres because of the propagation of current loops into the volume conductor surrounding the fibre. The penetration of such currents into other fibres was increased during the period of depolarization of the latter when they were excited. The potentials of neighbouring fibres, in agreement with the theory of recording potentials in a volume conductor, were recorded as two- or three-phase changes (plus–minus–plus), which were many times weaker than the investigated fibre's own potentials.

Translated by J. DAINTY

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AN INVESTIGATION OF THE TIME RELATIONS OF THE ACTION POTENTIAL AND IMPEDANCE CHANGES ON EXCITATION IN THE FROG NERVE*

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(*Received* 18 *December* 1956)

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THE study of the quantitative laws of the electric current flow through the nerve conductor [1–7] has established the wide possibilities of using the electrical conductivity method for settling some problems concerning the mechanism of excitation. At the same time there are also studies of great interest in which other electrophysiological methods are used, such as recording the action potential and action current [8–11]. Most of the latter studies, with parallel recording of impedance changes and the action potential, support the validity of the basic position of present-day membrane theory [12].

One of the important problems in these studies, devoted to investigation of impedance changes on excitation, concerns the time relations in the first stages of the action potential and the changes in electrical conductivity. The first studies in this direction led to a somewhat unexpected result. Thus, the experimental results obtained by Cole and Curtis, in studies on the alga *Nitella* and on the giant axon of the squid *Loligo*, lead to the conclusion that the changes in electrical conductivity lag behind the action potential. Most authors have not especially aimed at a detailed investigation of this question. Indeed, the fact of the action potential leading the impedance changes is discussed by some authors as one of the serious objections to the proposed membrane theory of excitation [13]. This circumstance already indicates the importance of this problem. Since the effect has been obtained on algae and unmyelinated fibres of invertebrates, we decided to study this question on myelinated fibres of the frog in conditions of a whole nerve trunk. A "low-lag", highly sensitive arrangement was necessary in order to achieve this aim.

In the present communication we describe the apparatus we have produced and some preliminary results of the investigations.

DESCRIPTION OF APPARATUS

We used a bridge method (with an oscillograph as the null instrument) as the most convenient and accurate method of measuring rapid changes in the complex resistance of biological specimens [6, 7]. A block diagram of the arrangement is shown in Fig. 1.

Fundamentally the construction of the bridge part of the arrangement is based on the principle of a bridge with a tuned output. The application at the output of the bridge of a resonance system gives the possibility of increasing the out-of-balance

* Biofizika **2**: No. 4, 417–426, 1957 [Reprint Order No. BIO 52].

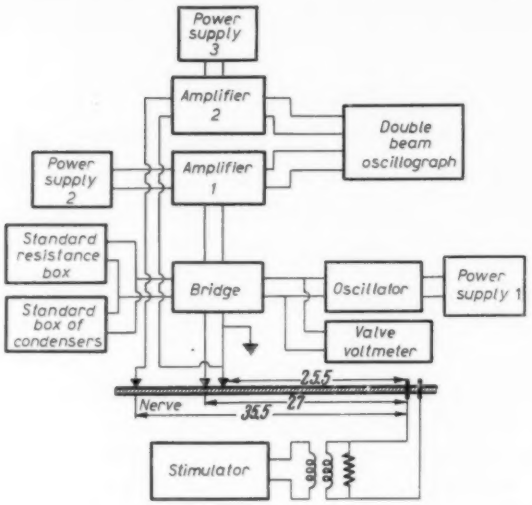

FIG. 1. Block diagram of the arrangement for simultaneous recording of the action potential and impedance changes on excitation of excitable tissue.

voltage by a factor Q. In this connexion the signal–noise ratio at the bridge output is also increased Q times; this is very important for obtaining a highly sensitive arrangement. The bridge part of the set-up allows measurement of the resistive (R) and capacitative (C) components of the impedance of the specimen according to a standard method of substitution.

The bridge was supplied with a sinusoidal voltage by an r.c. generator with zero phase shift. Such generators possess a wide frequency range, a great frequency stability and little non-linear distortion (this latter is very important in work with a highly sensitive bridge). The amplitude of the voltage of the generator was stabilized by negative feed-back, using a thermistor. The generator supply was obtained from a rectifier with an electronically stabilized voltage (power supply 1).

The value of the sinusoidal voltage was measured by a valve-voltmeter type VKS-7b.

As a null-indicator of the bridge balance one beam of a double-beam oscillograph type 2KO/721 was used with auxiliary amplifier 1.

Amplifier 1 was necessary so as to guarantee high sensitivity of the arrangement. The bridge was balanced only at the fundamental frequency the voltage of which was therefore low at the instant of balance. But the voltages of the higher harmonies were comparatively large, so that amplifier 1 was required to amplify selectively the fundamental frequency. To satisfy this requirement the instrument was a two-cascade resonant amplifier. The sensitivity of amplifier plus oscillograph was of the order of 10 μV for clear separation of the signal of the unbalanced bridge from the noise. In order to decrease interference the amplifier was supplied from accumulators (power supply 2).

The arrangement we have just described enabled us to carry out measurements of the impedance at any frequency in the range 10–1000 kc/s. At the same time the

resistive component of the impedance can lie between 1000 and 300,000 Ω, and the capacitative component from 5–10 to a few thousand pF. The sensitivity of the arrangement, measured in millimetres deflexion of the oscillograph beam when the resistive (or capacitative) component is changed, allows one to measure with confidence a resistance change of the order of 0·01 per cent.

For leading off and recording the action potentials a second channel is used, consisting of amplifier 2, its power supply 3 and the second beam of the oscillograph type 2KO/721. The action potential amplifier is a three-cascade voltage amplifier with resistance–capacity coupling. The amplifier has a balanced input circuit. The sensitivity of this channel is 27 mm/mV.

In order to establish the exact time relations and to obtain a stationary pattern on the oscillograph screen, the sweeps of both oscillograph beams have a common sweep voltage and are synchronized with the process being investigated. Synchronization is achieved by pulses which, in the stimulator type GRAKH, start off the rectangular stimulation pulses. Such a pulse coincides in time with the front edge of the rectangular pulse which is used for stimulation.

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METHODS

In this work we have used as our material the common trunk of the sciatic nerve of the frog *Rana temporaria*. Before putting the nerve into the chamber, its surface was carefully dried with filter paper. The hermetically sealed chamber was made of Plexiglas. The internal dimensions of the chamber were: length 46 mm, breadth 3·5 mm, height 6 mm. Through the side walls of the chamber (at a height of 3 mm from the bottom) 20 0·3 mm diameter platinum electrodes were passed, at a distance of 1·5–2 mm from each other. Thus the nerve trunk was lying, as a whole, only on the electrodes.

In our experiments the impedance electrodes were 25·5 and 27 cm distant from the stimulating electrodes and the electrodes recording the action potential were at 25·5 and 35·5 mm.

During the whole experiment the nerve remained in the chamber; the condition of the nerve was determined by its excitability and the maximum value of the action potentials, and also by the change in electrical conductivity. It must be noted that in 3–6 hr, the time the experiment usually lasted, the above properties changed up to 5 per cent. In the chamber conditions at a temperature of 17–20° the nerve was able to respond to stimuli for 2–3 days.

The experiments began by determining the maximum strength of stimulation for the given nerve. Then through a definite piece of the nerve, included between the impedance electrodes, an alternating sinusoidal current of frequency 35–70 kc/s was passed. This gave a voltage at the bridge input of 100–200 mV. At the frequencies we used these voltages were many times less than threshold. By means of standards of capacity and resistance a balance was obtained as judged by the minimum width of the beam on the oscillograph screen. Through stimulating electrodes were sent rectangular pulses of maximum strength, of frequency 50–70 sec and duration 50–100 μsec.

Because of the sweep synchronization by pulses from the generator type GRAKH, a stationary pattern which was then photographed was established on the oscillograph

screen. The upper beam was used for receiving the impedance changes on the screen, the lower for recording the action potential. Photographic recording of these two processes took place either simultaneously or separately.

We judged the time relations between the curve of the action potential and the curve of the impedance changes by the difference of their latent periods, which were measured by that part of the beam from its origin to the appearance of the effects. We used as time marks, in most cases, the sinusoidal current of frequency 35 kc/s, which passed through the indicator channel.

EXPERIMENTAL MATERIAL

A number of authors point out that those changes in electrical conductivity which occur when some materials (algae, unmyelinated nerves, muscles) are excited, could scarcely be observed in myelinated fibres of vertebrates because the overwhelming part of the resistance resides in the inactive myelin sheath [14, 15]. But there were indications in the literature as to the possibility of recording changes in

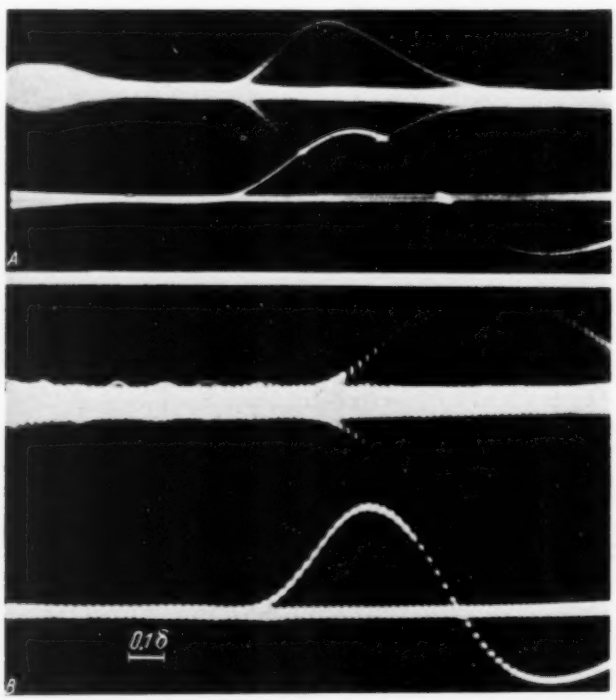

FIG. 2. *A:* simultaneous recording of the action potential and impedance changes on nerve excitation. The high-frequency pick-up in a similar recording (see text) was artificially suppressed, but at the same time the true time relations of the spike were distorted. *B:* recordings of impedance changes (upper curve) and action potential (lower curve). The spike-recording channel did not add any kind of distortion (see text). The bridge was supplied with a frequency of 35 kc/s. The impedance changes began 260–270 μsec later than the action potential. The experiments took place at a temperature of 19–20°.

the resistance of the common trunk of the sciatic nerve of the frog when it is stimulated.

In our first experiments we were satisfied that, thanks to the high sensitivity of our apparatus, it was possible to detect, in a sufficiently clear form, the impedance changes at the instant of excitation. In Fig. 2 an oscillogram is shown of the simultaneous recording of the action potential and impedance changes at the instant of excitation. As one should have expected, with the material we used, the impedance changes at the instant of excitation were very small. From a series of experiments it was clear that the relative decrease of the resistive component of the impedance varied between 0·03 and 0·10 per cent, and the capacity decrease between 0·1 and 0·3 per cent. We found the impedance changes at the instant of excitation by compensating the maximum value of the effect, in a similar manner to Cole and Curtis (Fig. 3). The fact that in our experiments the percentage capacitative changes were greater than the ohmic should be noted. This fact is clearly connected with our conditions of studying the whole nerve trunk.

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Simultaneous photographic recording of the impedance changes and the action potential appeared inconvenient because of the mutual interference of the two

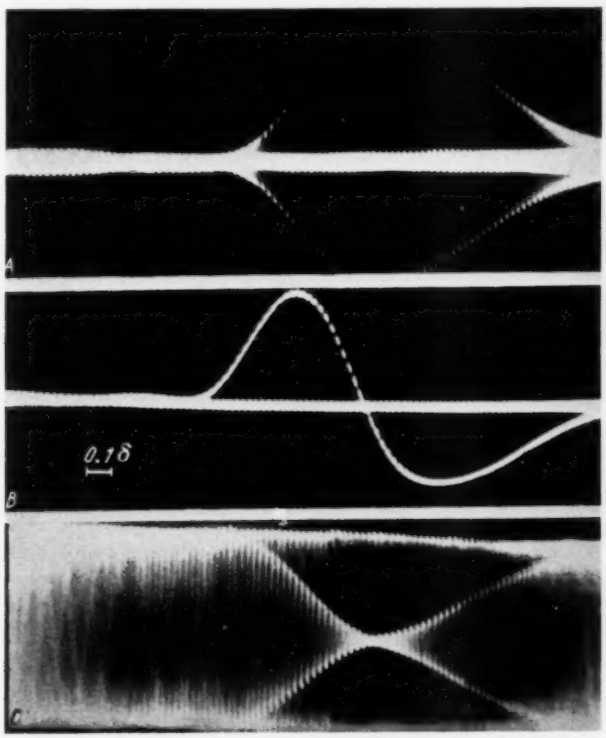

FIG. 3. Record of the impedance changes *A* and the action potential *B* on nerve excitation. In *C* the instant of compensation of the maximum amplitude of the impedance changes is recorded in order to determine their magnitude. The experiments were carried out at a temperature of 19–20°. The bridge was supplied at 35 kc/s.

channels (the high-frequency sinusoidal current of the indicator channel was superposed on the action potential). This was because we used a biological amplifier with a broad band-width. We had to apply a method of alternate switching-on of the processes for photographic recording without changing the sweep (Figs. 2 and 3).

In Figs. 2 and 3 oscillograms are shown for a series of experiments on the determination of the time relations of the curve of impedance changes and the action potential curve (frequency 35 kc/s). From these photographs and also from Table 1, in which the results of this series of experiments are summarized, it is clear that the impedance changes during the passage of the excitation process lagged behind the beginning of the action potential by 260–340 μsec. On average, for the whole experimental series, this time equalled 280 μsec.

TABLE 1. LATENT PERIOD IN μsec FOR ACTION POTENTIALS AND IMPEDANCE CHANGES

Latent period	Number of experiment							
	1	2	3	4	5	6	7	8
Action potential	840	810	720	780	870	960	880	920
Impedance changes	1100	1070	980	1040	1130	1300	1160	1200

NOTE: In this table the averaged results of eight experiments are given. The experiments were carried out at a temperature of 19–20°.

Theoretical calculations of the time of passage of the processes through both channels led us to the conclusion that the lag of the impedance changes might be dependent on the features of the channels themselves.

The passage of pulses through an amplifier is accompanied by distortions which can be characterized by two parameters: the delay time (t_d) and the rise time (t_r). By the delay time is understood that time between the beginning of the voltage pulse at the amplifier input and the instant of reaching half-maximum amplitude at the amplifier output. By the rise time is understood that time for growth from 0·1 to 0·9 times the maximum amplitude at the output of the amplifier. The delay time and the rise time are determined by the kind of circuit of the amplifier and its band-width. In the channel recording the action potential a resistance coupled three-stage amplifier is used with a band-width of 25,000 c/s. Theoretical calculations of t_d and t_r for such a circuit [17] give the values $t_d \simeq 9$ μsec and $t_r \simeq 11$ μsec.

In the indicator channel a two-stage resonant amplifier was used with a band-width of 1500 c/s at 35 kc/s. On the basis of theoretical calculations for such an amplifier, $t_d \simeq 230$ μsec and $t_r \simeq 450$ μsec.

These theoretical calculations show that the distortions connected with the action potential recording are insignificant and can be neglected, whereas the distortions in the indicator channel are considerable and must be allowed for.

In order to clear up the true time relations between the impedance changes and the action potential it was necessary to decrease the delay time in the indicator channel. It seemed possible to do this at the expense of an increase in the amplifier's band-width. But an expansion of the band-width at 35 kc/s would lead to an abrupt decrease in the amplification of the indicator channel, to an increase in the noise and accordingly to

the impossibility of recording impedance changes at the output. We therefore obtained the expansion of the band-width by transferring to a higher frequency (70 kc/s) for driving the bridge. At this frequency the band-width of the resonance amplifier increased to 3150 c/s, which should have decreased the delay time and the growth time by a factor of more than two.

In order to verify this an experimental study of the behaviour of the indicator channel was carried out with rectangular voltage pulses while supplying the bridge with frequencies of 35 and 70 kc/s.

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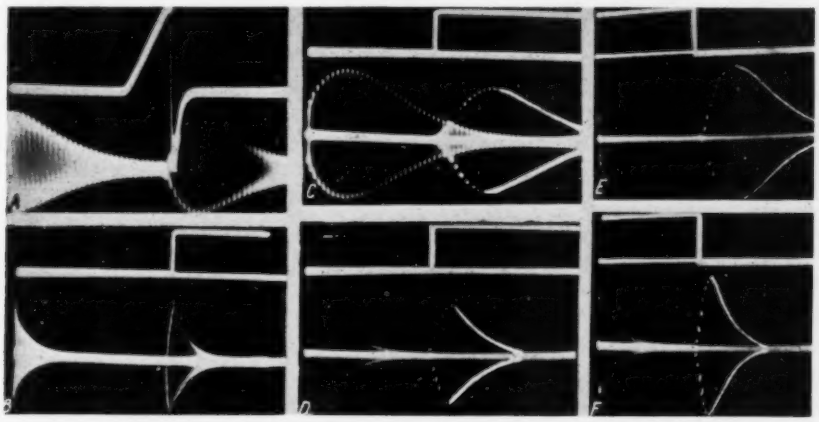

FIG. 4. *A*: behaviour of the indicator channel to a saw-toothed externally applied voltage. Bridge supplied at 35 kc/s. *B*: behaviour of indicator channel to a rectangular-shaped externally applied voltage without amplifier 1. Frequency: 35 kc/s. *C, D, E, F*—behaviour of indicator channel at making (*C, D*) and breaking (*E, F*) of an externally applied rectangular voltage. The maximum amplitude is reached more slowly at a frequency of 35 kc/s (*C, E*) than at 70 kc/s (*D, F*).

From Fig. 4, *C, D, E, F*, it is clear that at a frequency of 70 kc/s the delay time decreased, as compared with it at 35 kc/s, by a factor of more than two.

In Fig. 5, for comparison, the impedance changes recorded on one and the same nerve at 35 and 70 kc/s at shown in pairs. The measurements of the latent period from the photographs of this series of experiments are summarized in Table 2.

The data obtained show that the latent period of the impedance changes at a frequency of 70 kc/s was shortened by 180–200 μsec as compared with the length of

TABLE 2. EFFECT OF FREQUENCY ON LATENT PERIOD OF IMPEDANCE CHANGES EXPRESSED IN μsec

Latent period of the impedance changes at:	Number of experiment						
	1	2	3	4	5	6	7
35 kc/s	1600	1610	1630	1680	1530	1485	1510
70 kc/s	1400	1400	1450	1470	1350	1305	1350

NOTE: In the table the averaged data of 7 experiments are given. The experiments were carried out at a temperature of 16–17°.

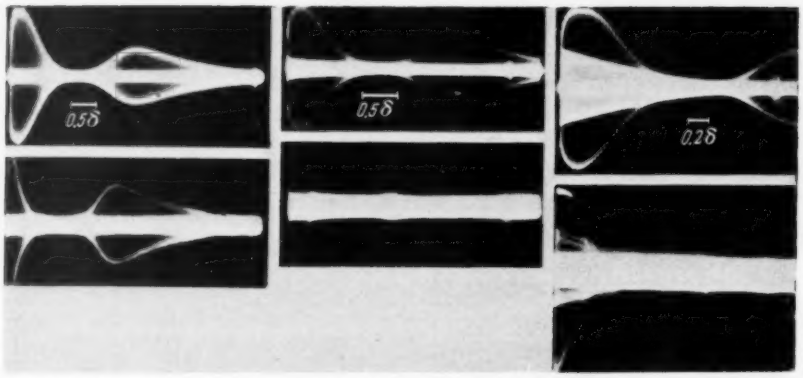

FIG. 5. Pairs of records of impedance changes on excitation at a frequency of 35 kc/s (upper curve) and 70 kc/s (lower curve) for three different nerves. The records were taken on different oscillograph sweeps. The impedance changes at a frequency of 70 kc/s begin 180–200 μsec earlier than at a frequency of 35 kc/s. The expansion at the beginning is connected with the stimulation artifact. The experiments took place at a temperature of 16–17°.

this period at a frequency of 35 kc/s. From the foregoing it is clear that this shortening of the latent period took place on account of the decrease in the delay time of the indicator channel amplifier. A diagram made up on the basis of the averaged data of a large number of experiments (Fig. 6) shows that the time of lag of the impedance changes behind the action potential also decreases by 180–200 μsec (see curves 1 and 2). In the absence of distortions in the indicator channel the curve of impedance would occupy the position shown, broken, in Fig. 6 (curve 3), and its origin would coincide in time with the origin of the action potential curve. Curve 3 can be obtained from curve 2 by "subtangent analysis" [18]. In this graphical scheme the amplitudes

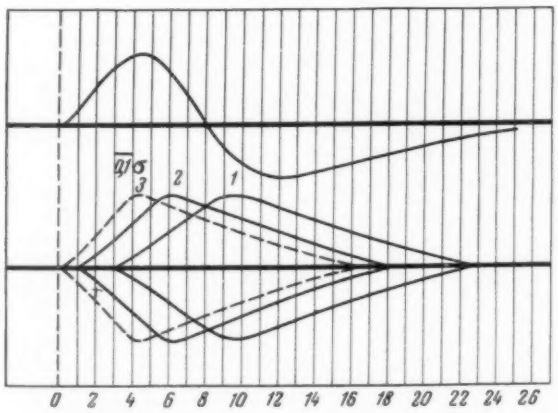

FIG. 6. Diagram of the time relations of the action potential (upper curve) and impedance changes (lower curve), composed on the basis of the averaged results of a great number of experiments. Curve 1 corresponds to the record of the changes of impedance with time at frequency of 35 kc/s. Curve 2: the same at 70 kc/s. Curve 3 (broken): ideal case in the absence of distortion in the indicator channel

of the impedance changes are equalized. Only the time relations are preserved in their true form.

The increase of the band-width of the indicator channel simultaneously decreased the distortion in shape of the curve of impedance changes. This was expressed by a decrease of the time of attainment of maximum amplitude. On the basis of the averaged results of the same experiments Table 3 was compiled showing the time of attainment of the maximum amplitude of the impedance changes at the frequencies 35 and 70 kc/s. For comparison the time of attainment of maximum amplitude of the action potential is shown. A comparison of these values proves that the smaller the distortion in the indicator channel, the closer is the shape of the left-hand side of the curve of impedance changes to the shape of the action potential. For the ideal curve, shown broken in Fig. 6, the shape of the left-hand side of the curve of impedance changes coincides with the curve of the action potential.

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TABLE 3. RISE TIME IN μsec OF IMPEDANCE CHANGES AND ACTION POTENTIALS

Rise time	Number of experiment			
	1	2	3	4
Impedance changes at 35 kc/s	650	640	650	675
Impedance changes at 70 kc/s	520	505	530	535
Action potential	435	406	464	464

NOTE: In the table the averaged data of 4 experiments are given. The experiments took place at a temperature of 16–17°.

The mean time of the latent period for the action potential, calculated from the data of Table 1, equalled 850 μsec. In our experiments the distance between the stimulating and the first recording electrodes equalled 25·5 mm. From these facts the speed of conduction of the wave of excitation can be taken as $\simeq$ 30 m/sec (at 19–20°). Thus the latent period of the action potential, which we compare with the latent period of the impedance changes, is determined for the fastest conducting fibres of the nerve trunk (group A).

CONCLUSIONS

The coincidence of the time of the appearance of impedance changes and the action potential and the similar course of their curves at the beginning, allow us to suggest that both these curves are different features of one and the same process, taking place in the nerve fibres during excitation. The coincidence in time of the development of the action potential and the impedance changes shows the correctness of the conclusions of the advocates of that point of view, according to which excitation and the appearance of the action potential are due to a change in the permeability of the active surface structures of the nerve fibre.

Translated by J. DAINTY

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THE NATURE OF THE INFORMATION ARRIVING AT THE BRAIN BY ONE NERVE FIBRE FROM TWO RETINAL RECEPTORS IN THE FROG*

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(*Received* 15 *November* 1957)

THE question as to the nature of information and its elaboration in the different parts of the nervous system is one of the most important in the physiology of the nervous system. It is known that the magnitude of the nervous impulse [1, 2] spreading along a single nerve fibre does not depend on the strength of stimulus ("all or none" law). Nevertheless, information as to the strength of the stimulus can be transmitted by a single nerve fibre. The response to a stronger stimulus is a volley of a greater number of nerve impulses.

Adrian† and Bongard and Smirnov [3, 4] have shown that there is reason for believing that a single nerve fibre can transmit information not only as to the strength, but also as to the nature, of the stimulus. In particular it follows from the work of Bongard and Smirnov [4] that a single ganglion cell of the frog's retina passes information to the brain regarding change of stimulation of both rods and cones. Such information cannot be furnished only by the number of nerve impulses in the volley, since this number depends on the intensity of the flash. Consequently it might be

* Biofizika **2:** No. 4, 427–430, 1957 [Reprint Order No. BIO 53].

† In a lecture before a meeting of the Academy of Sciences of the U.S.S.R. in 1956, Adrian reported observations on the difference in information concerning the "scent" of various substances.

expected that information as to the colour might be given by the nature of the distribution of the impulses in time in each volley signalizing change in the illumination.

The object of our work has been the study of the character of the temporal distribution of the nervous impulses and the dependence of this distribution on the intensity and colour of the stimulating light. The elucidation of this question is complicated by the circumstance that the nature of the information sent from the eye to the brain depends, as Iarbus, Bongard and Smirnov have shown, not only on the nature of the stimulating light, but also on the previous condition of the retina.

In order to ensure constant conditions of experiment we used the periodic switching on and off of a red or blue light. The light from a "point" incandescent lamp passed through a filter, a shutter and objective, and illuminated uniformly the whole retina of an isolated frog's eye, or a small part of the retina, the diameter of the spot being 0·1–0·2 mm. We obtained punctate illumination with the help of a movable lens or light-conductor and could direct it to any desired part of the retina. The nerve impulses from a single ganglion cell of the retina were led off by liquid electrodes of 10–20μ diameter. The method of preparation of the liquid electrodes has been described by Byzov [5]. Amplification, recording and counting of the nervous impulses was carried out with the aid of a Type 0-B-49 apparatus.* A diagram of the experimental arrangement of the preparation in the experiment is given in Fig. 1. In elucidating the change of rhythm occasioned by the change of

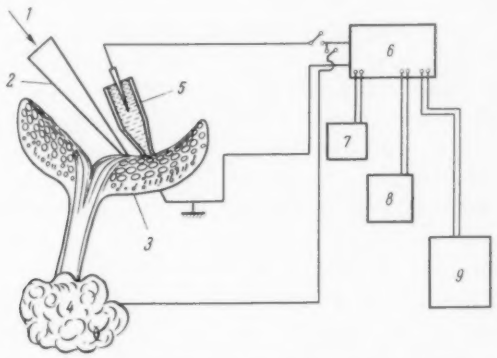

FIG. 1. (1)—light; (2)—light conductor; (3) isolated eye preparation; (4)—brain; (5)—microelectrode; (6)—amplifier type D; (7)—cathode-ray oscillograph; (8)—loop oscillograph; (9)—counting apparatus PS-64.

light, we strove to obtain a constant number of impulses transmitted in experiments along a single fibre during the first 0·1–0·2 sec after switching on either a red or a blue light. For this purpose we chose corresponding intensities of red and blue light.

In our experiments we observed, as have other authors [4, 6], ganglion cells responding to the switching off of the light (off-effect), to the switching on of the light (on-effect), and to both switching on and off (on–off-effect). With uniform illumination a considerable fraction of the single fibres of a fresh preparation of the

* We note that the O-B-49 apparatus was used by E. S. Shepot'eva and S. I. Ardashnikov in 1954.

frog's retina transmits signals mostly of the diminution of the illumination. In these fibres the difference in the rhythm of the impulses arising on switching on and switching off of the blue and the red light is not great. According to preliminary results, under these circumstances when the fibre does not give a clear difference of rhythm the same number of impulses continues for a shorter period of time after switching off the red light than after switching off the blue.

The on-fibres activated only by switching on the light are rare (approximately 1 in 100); we did observe some such fibres. In these fibres the difference between responses to switching on the red and the blue lights was quite clear. After switching on the blue light (Fig. 2a), over wide limits of intensity single impulses were observed for a prolonged period of time. At first they were of high frequency, the frequency then periodically diminished and increased; with increase of intensity the frequency increased. The duration of the volley of impulses was more than 2 min with a sufficiently powerful blue light. After switching on the red light (Fig. 2b) there arises a short volley of impulses lasting less than 0·5 sec. The volley contains from 5 to 20 impulses. The number of impulses in the volley is different for different cells, and increases with increase of intensity of the light.

The effect described is observed in an insignificant fraction of the fibres. It is clear that with the help of these fibres it is not possible to transmit information as to the details of a visual image; it is, however, not impossible that information as to colour during uniform illumination of the retina could be transmitted by such an economical means. Information as to the details of the visual image must be transmitted by a considerable fraction of the fibres. It might be expected that transmission of this information of the details of a coloured image is reflected by the individual fibres in the form of such codes as arise on uniform illumination of the whole retina in the on-fibres. To create "coloured details" on the retina we made use of microbundles of light rays. Parts of the retina were illuminated by a coloured spot at various distances from the microelectrode.

The receptor field of a ganglion cell of the retina has been studied by Kuffler and Barlow [6, 7]. According to our observations this receptor field may sometimes extend in the frog over more than 5 mm^2.

We were interested in the relation between the nature of the code arising in response to switching on the light and the position of the coloured spot. It proved to be possible to find for the majority of the ganglion cells such a position of the microspot (in relation to the electrode) that the fibre gives a predominantly on-effect.* In this, the same difference of rhythm of impulses was observed in response to switching on the red and the blue light as was observed for the on-fibres of the uniformly illuminated retina (Fig. 3a and 3b). The difference between the blue and the red codes was the more distinct, the more intense the light. It consisted not only of a different duration of the signals but of peculiarities of the rhythm. At low intensities the difference in the rhythm was greater than the difference in duration of the signal.

It seems to us that the difference in rhythm may be described as follows. Let us denote by " active group " one impulse, or a batch of impulses, the interval between which does not exceed 0·02 sec. The experiments show that in the volley of nerve

* The transformation of an off-fibre to an on-off and to an on-fibre on shifting the microspot on the retina was first observed by Kuffler [6].

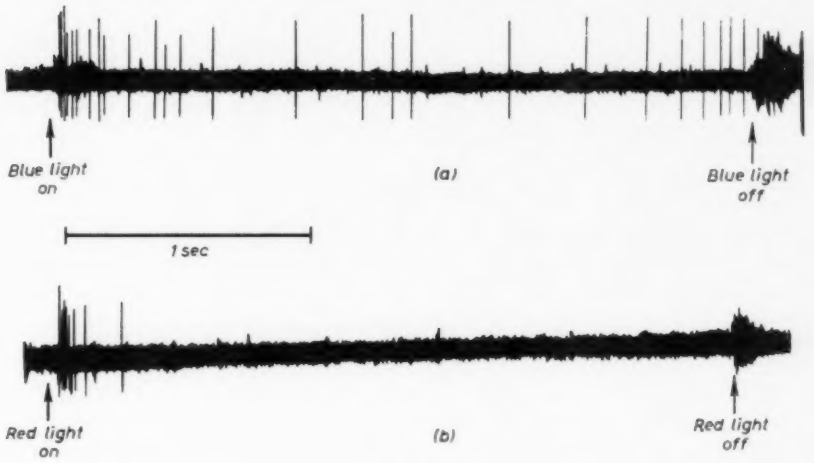
Blue light
on
(a)
Blue light
off
1 sec
Red light
on
(b)
Red light
off

FIG. 2.

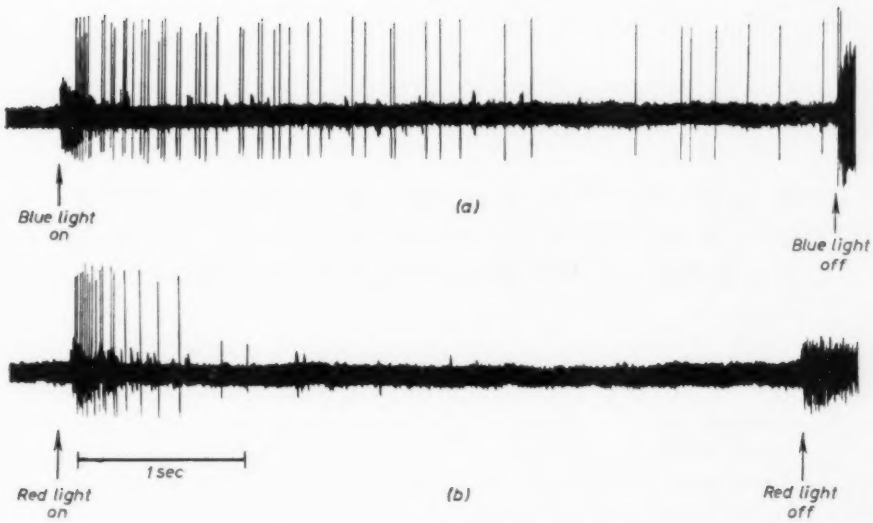
Blue light
on
(a)
Blue light
off
1 sec
Red light
on
(b)
Red light
off

FIG. 3.

impulses arising from red light the intervals between the active groups either increase or remain constant. In the volley of impulses arising from blue light these intervals periodically diminish and increase.

The difference with which transmission of information as to colour is associated can be elucidated by the aid of the technique of conditioned reflexes; a conditioned reflex to a blue and to a red light is elaborated, and then in place of the light electric impulses of the appropriate rhythm are applied to the optic nerve.

CONCLUSIONS

(1) In single on-fibres a difference of rhythm of the nerve impulses transmitted to the brain by a single ganglion cell of the retina was observed between the responses to switching on a blue and a red light.

(2) With the help of a coloured microspot the same difference was obtained for a considerable fraction of the fibres.

(3) The experiments carried out confirm the hypothesis that a single nerve fibre can transmit multidimensional information.

The author expresses his thanks to I. M. Weinzweig for help with the work and to N. D. Nieberg for a full discussion.

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CHANGES IN THE ELECTRICAL SENSITIVITY OF THE EYE AFTER ILLUMINATION WITH INTENSE FLASHES OF DIFFERENT COLOURS*

P. A. KORZUN

(*Received* 6 *February* 1957)

ELECTRICAL irritation of the eye gives rise to a peculiar light sensation (phosphene). Some authors consider that an electric current of threshold intensity acts on the nerve elements of the retina [1–5 and others]. Others maintain that in threshold electric phosphene the optic nerve fibres are affected [6, 7]. A number of workers [5, 6, 8] have established that the electrical sensitivity of the eye diminishes in the dark (i.e., the threshold rises). The sensitivity also changes in response to various drugs and concomitant irritants as well as in cases of disease or damage to the eye [3–5].

* Biofizika **2:** No. 4, 431–440, 1957 [Reprint Order No. BIO 541

From 1949 to the present time the most systematic study of phosphene has been conducted by the Japanese scientist Motokawa and his co-workers [9–12], using an original technique. This procedure involves the following: after 15–20 min of adaptation to darkness, the electrical sensitivity of the eye to a direct current lasting 100 msec is determined. Let this sensitivity be E_0. The eye is then illuminated with a short flash and its sensitivity determined 1, 2, 3, etc. sec after the termination of the flash. Sensitivity rises and by a certain time equals E. Then the relative change in the electrical sensitivity is $e = 100\,(E-E_0)/E_0$.* It has been shown that e depends on the time which had elapsed since the termination of the flash, i.e. $e = f(t)$. It rises at first, reaches a maximum, and falls to zero. The time in which e reaches its maximum depends on the colour of the light flash, and on the retinal site being illuminated. Thus in illuminating the fovea centralis with red, green, white or blue light e reaches its maximum 1, 2 and 3 sec, respectively, after the termination of the flash. Neither the duration (up to 4 sec) nor the intensity (0·9–1470 lx) of the flash have any effect on the position of the maximum. When the extreme periphery of the retina (40° or more from the fovea centralis) is illuminated, e reaches its maximum between 1 and 2 sec after the end of the flash, for all colours. Illumination of sites intermediate between the fovea centralis and the extreme periphery of the retina with red, blue and green light produces the same relative changes of electrical sensitivity as does illumination of the fovea centralis, provided the intensity of the flashes exceeds the threshold of receptivity of the lamp. Illumination with white light results in two and three maxima of e when sites 15°, 40° and 5° from the fovea centralis respectively were illuminated.

The authors have used their technique in studies of numerous problems of vision (colour and twilight vision, light and colour contrast, optical illusions, determination of the boundaries of the field of vision, etc.). In spite of the interest of the problems raised and of the fact that the work [9–12] has been published in many languages, nobody else has so far used the method for studying vision problems.

The purpose of the present work, carried out under the guidance of Prof. N. T. Fedorov, has been, apart from the reproduction of certain findings of the Motokawa school, the study of electrical sensitivity changes in the eye after short and sufficiently intense illuminations with light of different colours.

METHOD

Apparatus

Single impulses of exponential form and of 40 msec duration were used for the electrical irritation of the eye. A slight modification in the circuit of an apparatus used for electrostimulation of muscle (ASM–2) ensured obtaining a single impulse instead of a series of impulses produced by the apparatus. This modified circuit is shown in Fig. 1. A block of impulse modulators cut off in the apparatus; an electromechanical switch B_2 was included in the circuit of the square-wave pulse generators (multivibrators). This switch closed the multivibrator network circuit for 60 msec, thus ensuring the production of only one impulse lasting 40 msec; this was checked by the Siemens-Galske loop oscillograph. The pulse form was controlled by a cathode-ray oscillograph. When the contact is interrupted by $_2$,B

* Motokawa has designated this ξ, but for convenience of printing we have substituted e.

the multivibrator does not function and the apparatus produces galvanic current. To prevent its passage into the subject, a low-frequency filter consisting of a choke L_3 and a condenser C_{10} of 10 μF capacity were inserted at the outlet of the apparatus. The circuit of the subject starts with the exit potentiometer in the cathode circuit of the valve 6P3, making it possible to regulate the magnitude of the voltage and consequently that of the pulse. This circuit includes a coupling condenser C_{10}, an additional resistance R_{64}, electrodes, a microammeter and a voltmeter. A "Norma" microammeter provided a check of the current strength of the pulse at the moment of closing the multivibrator network circuit; a voltmeter of the MVA47/5 magneto-electric type, of the highest precision, determined the magnitude of the constant component of the potential when the multivibrator was switched off, and of the pulse potential when it was switched on. The resistance of subject circuit was maintained by selection of the electrodes and the electrode paste, and by varying the additional resistance R_{64} (0–20kΩ). The electrodes were cup-shaped (bottom diameter 3 cm, height 1·5 cm). Their inner surfaces were coated with silver chloride, and they were filled with an electrode paste prepared from finely divided asbestos saturated with a mixture of 10 per cent glycerol and 90 per cent of a 1 per cent solution of sodium chloride. Thus, electrode polarization and drying of the paste were almost completely prevented, and a close contact with the skin was ensured.

Light source

In the first series of experiments, an incandescent gas-filled lamp was used, of 275 W, which provided an illumination of the pupil (using light filters and a distance from the observer to the light source of 1 m) with about 22 lx. A red, a green, a blue and a neutral filter were found which experimentally gave approximately identical illumination of the pupil.

In the second series, an impulse lamp ISS–250 served as a light source, giving an illumination of the pupil of about 10^5 lx under the same experimental conditions.

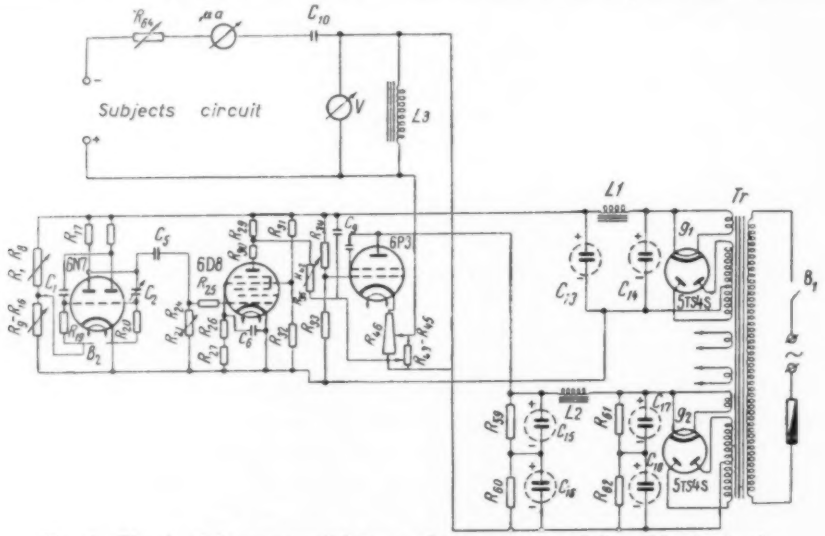

FIG. 1. Circuit of the exponential form pulse generator and the subject's circuit.

The duration of the flash was, according to the specification, 0·9 msec. The correctness of this was experimentally checked by a stroboscopic procedure. The circuit of this lamp is shown in Fig. 2.

According to data recently published [13], the impulse lamp used by us develops, during the flashes of light produced by the gaseous discharge, a colour temperature of up to 7000°K. In conformity with this, calculations from a curve of the energy distribution in the spectrum of our source were made for selecting light-filters of red (KS–11), green (ZS–2) and blue (SS–5) glass, giving approximately the same illumination (25×10^3 lx) of the pupil. The illumination was four times greater with the neutral filter. The curves of filter transmissions were determined with a spectrophotometer (SF–2M).

To ensure the safety of the experimental procedure, preliminary tests were carried out on rabbits, using an intensity of light 150–200 times greater than the working one. The author subjected himself to repeated illuminations (in the course of one sitting) with light 75–100 more intense than the working level. Careful ophthalmoscopy was performed at the eye clinic. Observations were carried out for three months. A considerable decrease of intensity, by means of increasing the distance and using filters, ensured the safety of the procedure. Control ophthalmoscopy was performed systematically in the course of the experiments.

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Methods of investigation

One man and three women, normal trichromates, 20–30 years of age, were subjected to the tests. These were carried out in a darkened room removed from sources of noise. The subject was 1 m away from the light source, which was kept in a box with an aperture of 3 cm × 3 cm for the light filters and thus was seen at a 1° 45′ angle of vision.

A red fixation point, 1 mm², permitted the illumination of different parts of the retina. The inactive electrode (+) was placed on the lower third of the right fore-arm and the active (−) one over the right upper rim of the eye socket. The electrodes were attached with a gauze or rubber bandage according to the subject's preference.

After 15–20 min of adaptation to darkness, the electrical sensitivity of the eye to an isolated exponential-form impulse (the inverse of threshold phosphene in volts

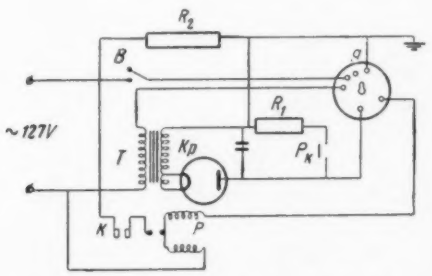

FIG. 2. Circuit of impulse lamp ISS-250.

$E_0 = 1/V_0$) was studied. A short illumination of one eye followed, and the electrical sensitivity was again measured 1 sec after cessation of illumination. If the voltage was above the threshold value, it fell considerably at first and fell, on approaching the threshold, by 3–5 per cent. This procedure was repeated several times after 8–10 min and 15–20 min adaptation in fovea centralis and in retinal periphery illumination respectively. The electrical sensitivity was regarded as established when, in two measurements, the subject once did not notice the phosphene and once barely registered it. The relative sensitivity of the eye $e = 100\,(E-E_0)/E_0$ was determined. Similarly e was determined for 2, 3, 4, etc. sec and the relationship $e = f(t)$ was established. The first two or three experiments served for orientation and training in determining thresholds. For training, 4 sec after the impulse being studied, a control one, lower than the threshold, was given. This was withdrawn in the course of training. One second before the impulse the subject was warned by the word "attention" or was told at which second the impulse was due. Two or three series of experiments on the illumination of a given retinal site with each colour were carried out with each subject, and the relationship sought was obtained from the mean values. The number of series of experiments was limited to two if the results of both were very close, but more often three or more series were performed. In the second series the time order of determining e after illumination was reversed—viz. 6, 5, 4, etc. sec—and in the third series it was random. Generally during the session (2–3 hr), the relative change in electrical sensitivity of the eye was determined for 1–6 sec after illumination. If e had to be determined for a longer period, a second contact was used sending out impulses 5 sec after the first.

The method described was used to study (1) changes in the electrical sensitivity of the eye after 2 sec illuminations of the retinal centre and periphery (15° from the fovea centralis), with light of different colours and low intensity (22 lx on the pupil), and (2) electrical sensitivity changes after illumination of the same sites with intense flashes of different colours.

RESULTS

Electrical sensitivity changes in the human eye after 2 sec illumination of the fovea centralis and the retinal periphery 15° from the fovea centralis with light of different colours and of low intensity

(1) After illumination of the fovea centralis with red light the relative rise in the electrical sensitivity of the eye reaches its maximum 1 sec, with green and white light 2 sec, and with blue light 3 sec, after the cessation of illumination. Fig. 3 shows the data on subject D.E.M. Each curve represents the results of two series of experiments. Time in seconds is plotted along the abscissa and e in percentages along the ordinate. In subject S.O.P. the relative rise in electrical sensitivity reached its maximum 2 sec, 3 sec and 4 sec after illumination with red, with green or white and with blue light respectively; i.e. there was a retardation of 1 sec in the appearance of the maxima (Fig. 4a). Each curve represents the results of three series of experiments.

(2) After illumination of retinal periphery (15° from the fovea centralis) the same, though less clearly marked, time sequence of reaching maxima for red, green, white and blue colours is obtained (Figs. 4b and 5). These figures also show that curves

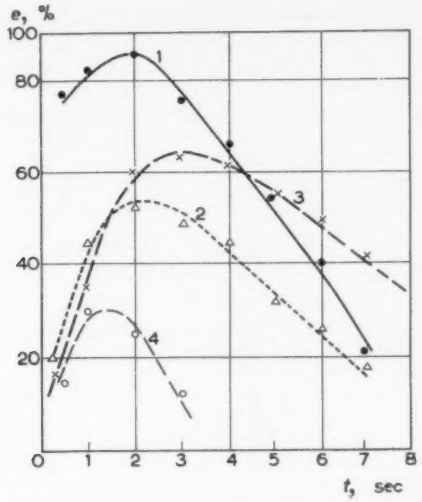

FIG. 3. Curves of the changes in electrical sensitivity of the eye (subject: D.E.M.) after 2 sec illuminations (22 lx on the pupil) of the fovea centralis. 1—white; 2—green; 3—blue; 4—red.

representing the relationship $e = f(t)$ decrease more slowly after the maximum than in foveal illumination, and even have a second small shoulder 6–7 sec after illumination with white light. The curve of the relative electrical sensitivity changes after blue light illumination in S.O.P. also has a more complex character. Four series of experiments were carried out in which were determined the changes in e on illumination of the same retinal site with blue light of a still lower intensity (10 lx on the pupil). The bottom curve in Fig. 4b gives the results of these measurements: it clearly shows two maxima: the first 2–3 sec, and the second 6–7 sec, after the illumination.

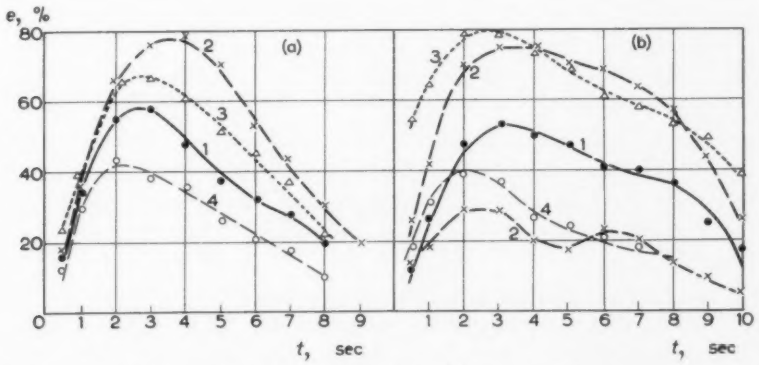

FIG. 4. Curves of changes in electrical sensitivity of the eye after 2 sec illuminations (22 lx on the pupil) of the centre (a) and of periphery (b) of the retina in S.O.P. 1—white; 2—blue; 3—green; 4—red.

(3) The relative rise in the electrical sensitivity of the eye is less after illumination with red light than with light of other colours.

Changes in the electrical sensitivity of the human eye after illumination of the fovea centralis and the retinal periphery 15° *from the fovea centralis with intense flashes of different colours*

(1) A significant (0·5–1·0 sec duration) initial fall, instead of relative rise, in electrical sensitivity was found to occur. It is more marked after illumination of the fovea centralis than of the retinal periphery. In both cases, it reaches its maximum after illumination with flashes of white and of blue light, as shown in Figs. 6–8. These show that there is no initial depression after illumination with flashes of red light. The maxima of relative rise in electrical sensitivity after illumination of the

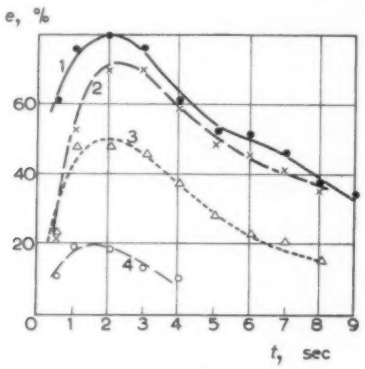

FIG. 5.

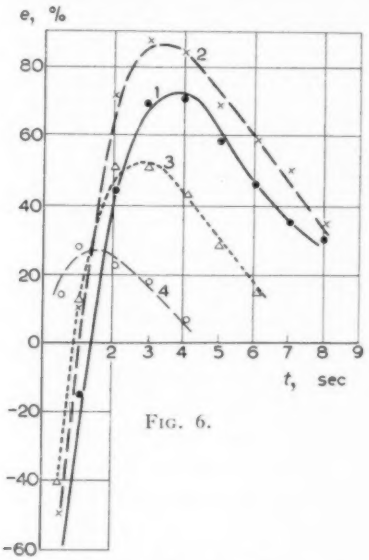

FIG. 6.

FIG. 5. Curves of the changes in electrical sensitivity of the eye after 2 sec illumination (22 lx on the pupil) of retinal periphery (15° from fovea centralis) in D.E.M. 1—white; 2—blue; 3—green; 4—red.

FIG. 6. Curves of the changes in electrical sensitivity of the eye after fovea centralis illumination with an impulse lamp (D.E.M.). 1—white; 2—blue; 3—green; 4—red.

fovea centralis with an impulse lamp are displaced along the time axis to the right by 0·5 or 1·0 sec—depending on the duration of the initial fall in *e* (Fig. 6).

(2) After illumination of retinal periphery, the relative rise in sensitivity reaches its maxima for red light after 1–2 sec, for green after 2–3 sec, for white and blue after 5–6 sec (Figs. 7–8).

Using the present method it is difficult to claim greater precision in finding the maxima for illumination of the retinal periphery with white or blue flashes. This is related to the prolonged rise in electrical sensitivity of the eye (15 sec or over) leading to fatigue of the subject and to fluctuations of the threshold.

(3) Figs. 7–8 show that the curves representing the relative changes in the

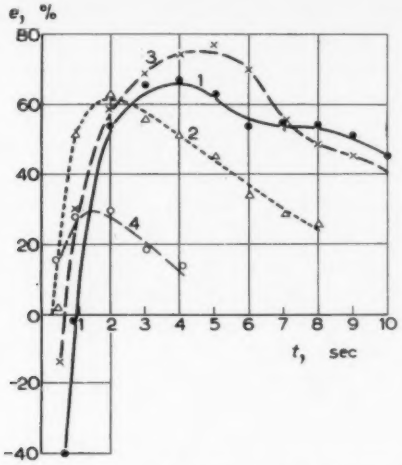

FIG. 7. Curves of the changes in electrical sensitivity of the eye after retinal periphery (15° from fovea centralis) illumination with an impulse lamp (D.E.M.). 1—white; 2—green; 3—blue; 4—red.

electrical sensitivity of the eye following retinal periphery illumination with white or blue light are of complex character: secondary maxima appear, their position on the time axis being dependent on the colour of the illumination and on individual peculiarities of the subjects. Thus, S.O.P. shows secondary maxima after illumination with blue, white and green light (Fig. 8), whilst D.E.M. does so only after white light illumination.

Experiments on other subjects gave analogous results and are not detailed here.

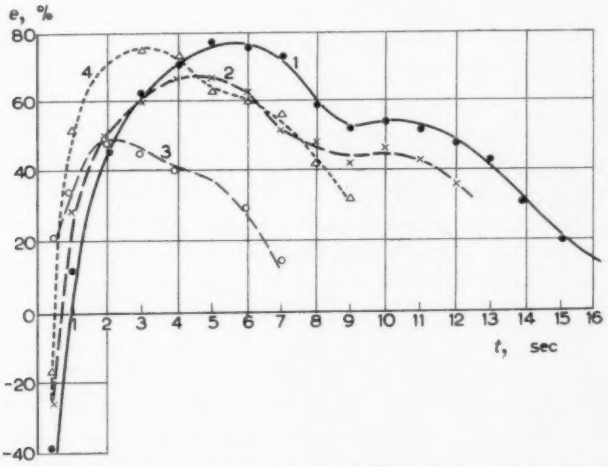

FIG. 8. Curves of the changes in electrical sensitivity of the eye after retinal periphery (15° from fovea centralis) illumination with an impulse lamp (S.O.P.). 1—white; 2—blue; 3—red; 4—green.

Curves showing the changes in the electrical sensitivity of S.O.P.'s eye after fovea centralis illumination by the impulse lamp are also not given, since they are basically similar to those shown in Fig. 6.

DISCUSSION

(1) It has been shown by Motokawa and by other workers that differences exist in the time of visual analyser reactions depending on the wavelength of light acting on the eye. Thus, Goto and Toida [14] have found in the isolated frog eye at 5–10°C that the wave *d* of the electroretinogram, representing the reaction to light extinction, consists of a number of waves. Selective inhibition revealed that the first wave is related to the extinction of red, the second of green and the third of blue light. The fourth wave is connected with the red reaction to light extinction.

Donner [15] has studied the pulse frequency of nerve elements in a cat retina under the action of light impulses of constant energy but different wavelengths. He has established that on–off elements for the long-wave (600μ) stimulus give maximum frequency after 0·15 sec, the 520μ after 0·22 sec and the 460μ after 0·30 sec. Under these conditions the on-elements have, apparently, one maximum.

Monnier and Dieterle [16] have established electrographically that the retino-cortical time is shortest under the action of red light stimuli on the eye, increasing with the shift of the light stimulus towards the short-wave end of the spectrum. Possibly the above facts indicate that processes in the visual analyser proceed at different speeds under the action of light stimuli of different wavelengths. These differences are apparently related to the functioning of all the components of the visual analyser, starting from the retinal receptors and finishing with the cerebral cortex.

(2) The initial fall in electrical sensitivity following illumination with intense light flashes is related to inhibitory processes in the visual analyser. This is confirmed by the electroretinogram findings, recorded during intense flashes of light. Thus Cob and Morton [17] have found that the e.r.8. in such cases consists of a large negative wave *a*, followed by the positive wave *b* overlaid by 4–6 small waves. They relate the large negative wave to the component R_{111} of the e.r.8 (according to Granit).

(3) There are some discrepancies between our findings and those of the Motokawa school [10, 15, 16].

Thus, according to Motokawa, after white light illumination of retinal periphery 15° from fovea centralis, there appear two maxima of *e*: the first at 1·5 sec and the second at 3 sec after the cessation of illumination. The author explains this by the preponderance in this retinal site of blue and yellow light receptors and the paucity of green light receptors. Our findings have shown that after white light illumination of this retinal site the second maximum is less marked and occurs 6–7 sec, whereas the first one occurs 2–3 sec, after cessation of illumination. In one of the subjects we have also observed two maxima after blue light illumination of a given retinal site (Fig. 4b) which occur after 2–3 sec and to 6–7 sec respectively. These maxima stand out particularly clearly on the reduction of the light intensity. They apparently characterize two processes which become additive with an increase in light intensity, thus making the maxima less clear-cut. Possibly this is due to the current form used in

electric stimulation of the eye. We have used exponential form impulses to which, according to Kurosawa [18], blue light and twilight receptors are hypersensitive. This evidently also explains the wave-like shape of the relative electrical sensitivity changes curve after illumination of the retinal periphery with white or blue flashes.

CONCLUSIONS

(1) After a 2 sec illumination of the eye with a source of light of low intensity, the curve, representing the dependence of the relative change in the electrical sensitivity of the eye on the time elapsed since the cessation of illumination ($e = f(t)$), has a characteristic shape: it rises to a maximum and then falls to zero. After illumination of the fovea centralis the maxima occur 1 sec after red, 2 sec after white and green and 3 sec after blue light illumination. The maxima are similarly distributed along the time axis after retinal periphery (15° from fovea centralis) illumination with light of different colours. It is possible that the characteristic positions of the maxima are related to the time differences in the course of perception of red, white, green and blue colours.

(2) After illumination of the above two retinal sites with high-intensity flashes of different colours, a significant fall instead of a rise in electrical sensitivity is observed. It is more pronounced in illuminations of fovea centralis than of retinal periphery and in both cases is most marked after white and blue, and is absent after red, illuminations. This phenomenon may be related to inhibitory processes in the visual analyzer.

(3) After retinal periphery (15° from fovea centralis) illumination with a light source of low intensity with neutral or blue filters, the curve reaches a maximum, falls slowly and then shows a second small rise after 6–7 sec. This second peak is more clearly revealed after retinal periphery illumination with an impulse lamp.

Translated by I. GORE

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METHODS OF ANALYSING SPECTROGRAMS OF VOWEL SOUND*

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(*Received* 15 *November* 1956)

No matter how speech-sound spectra are dealt with (by an observer or by an automatic differentiating apparatus) the problem is basically one of recognizing the sound and determining which phone to assign it to.

The difficulty lies in the fact that the spectra of a given phone show marked differences when pronounced by different subjects or in different sound combinations [1–7]. But the varied spectral characteristics of a given sound do not prevent its correct perception, which implies that not all the information contained in the spectrum is of use in recognizing the sound. This is shown by the fact that very marked artificial distortions of the sound spectrum have, under certain conditions, little effect on recognition [6, 8–10]. So the requirements to be applied to any method of analysing the spectra may be formulated as follows: (1) if possible the method should only extract the useful information; (2) the useful information should be extracted completely so that the sound can be recognized and its phonetic components determined.

We here consider two different methods for this purpose, which can be termed briefly the formant and envelope methods. The three vowels "a", "o" and "u" as separated from the words "khata", "toka" and "tupo" pronounced by 20 male announcers were used.†

THE FORMANT METHOD

A common method of describing sound spectra is to represent the spectrum as a combination of frequencies corresponding to formants. But in practice the method is very complicated to use because the way to find formants on the spectrograms and to determine their frequencies is unknown.

By formant is normally meant a group of the components of higher intensity. But there is no quantitative criterion for "higher intensity". Hence the separation of formants from spectra which are, as a rule, extremely irregular, becomes very arbitrary, on account of this lack of a definition. Different workers find very varied numbers of formants in the spectra of the same sound (from 1 to 5 or 6). An example is the formant composition of the Russian vowel sounds (see Table 1). Up to eight resonance regions can be detected [11] when the frequency characteristics of the vocal cavities are measured directly while various vowels are being pronounced. Naturally, so many formants are found that we may wonder whether they are necessary for recognition [7] and how the essential and inessential formants are differentiated.

† The spectrograms were obtained from the Research Institute of the Ministry of the Electronics Industry.

* Biofizika **2**: No. 4, 441–451, 1957 [Reprint Order No. BIO 55].

Formant frequency determination is no less complex. At least three methods exist, according to Flanagan [19]: Ochiai and Fukumura [5] used two. The simplest, involving no *a priori* assumptions, is to determine the frequency of the strongest component; but simple considerations show that the frequency at the maximum when the main frequency is high may differ substantially from that at which the vocal cavities are set (so that the resonant frequency remains to be determined). Thus, when the main frequency is 300 c/s the maximum may differ from the resonant by as much as ± 150 c/s.

Other methods of determining formant frequencies (i.e. from the mean or weighted mean frequency) require various items of *a priori* information, such as the Q's of the speech resonators or a knowledge of the weighting law. If this information is inadequate, or lacking, the determinations inevitably become very arbitrary.

As the formant compositions of speech sounds are difficult to determine from spectrograms other methods have been sought. Thus some workers [6, 7, 20, 21] have used synthetic sound perception to determine formant compositions. But when maximally simplified standard speech sound spectra have been found by synthesis the problem of evaluating the spectra of natural sounds is still unsolved. Having the spectrogram of a natural sound we have, as before, to analyse it to determine of which of the standard synthetic sounds it is mainly composed.

The table gives data on the formant compositions of Russian vowels derived from various sources and by various methods. It is clear that the data are largely incompatible; and a given frequency region might appear to correspond to different sounds.

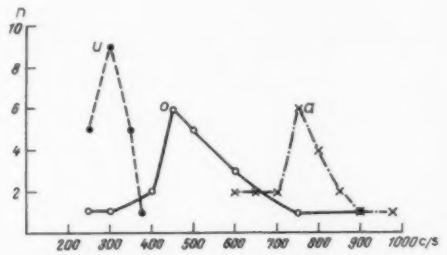

FIG. 1. Frequency distributions shown by the strongest components in "a", "o" and "u".

Fig. 1 shows the frequency curve for the first formants in "a", "o" and "u" derived from 60 spectrograms at our disposal (20 for each vowel). As formant frequency we have taken that of the strongest component (except for the second harmonic, which is neglected in the treatment). This method was chosen because although it is inadequate it is the least arbitrary. Fig. 1 shows that the frequency regions in which the maximum components of the various sounds fall overlap to a large extent. This corresponds with Potter and Steinberg's [2] and Peterson's [3] data, which were obtained by other methods of formant frequency determination (weighted mean and maximum on the formant enevelope).

So it is in some cases impossible to recognize a sound from data on absolute frequencies for formants alone. Potter and Steinberg have supposed that the auditory

system in man records formant frequency ratios as well as absolute frequencies, thereby providing another parameter for phone determinations. But the data on the perception of synthetic speech do not confirm this idea.

We thus conclude that the formant method of dealing with spectrograms is a very arbitrary one and gives insufficient information for differentiating sounds.

THE ENVELOPE METHOD

If we assume that the communication transmitted to the hearer is the frequency characteristics of the vocal cavities in the speaker it is evident that we get the maximum information about the phone pronounced if we take into account the envelope of the spectrum (spectral envelope methods of various kinds for differentiating phones have been proposed by Miasnikov [22], Smith [23] and Davis [24]). But we already know that the shape of the envelope varies considerably from one individual to another. So, to evaluate the possibilities of identifying spectrograms from their envelopes, the spectrograms must first be treated statistically, the mean shape of the envelope found for each phone and the deviations from the mean determined for each.

To work with envelopes we must represent them as some sort of series, as a sequence of numbers. In other words, the whole spectrum must be split up into bands and the height of the envelope (peak or mean) determined in each band.

In this work the frequency range 100–4000 c/s was split up into bands of 200 mel. This band-width was determined by our having 20 spectrograms for each phone with fundamental frequencies from 100 to 200 c/s, and a finer subdivision would not have enabled us to use all the material. The peak value was taken as the envelope characteristic to be used in the band (value in dB of the strongest component in the band). Thus, the preliminary treatment gave a collection of individual envelopes, each envelope being specified by 11 discrete values. To compute the mean envelope for each phone the individual envelopes were normalized by reducing the sums of the envelopes over the 11 bands to zero.

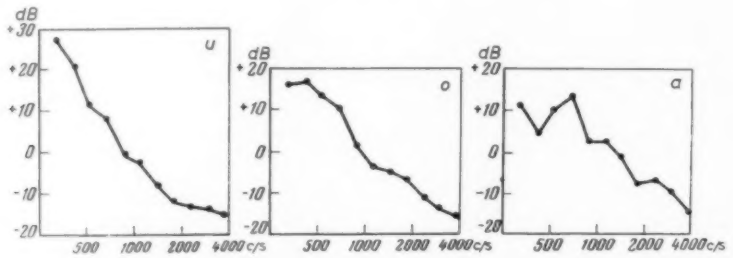

FIG. 2. Mean spectral envelopes for "a", "o" and "u".

Fig. 2 shows the mean envelopes for the phones. The shapes differ between the phones, but one naturally cannot say whether they are sufficiently different to the eye to ensure recognition of the sounds. To solve this problem it is essential to study the deviations from the mean, to determine the form of the deviation distribution and the mean square deviation. Study of the deviations in each band for each phone showed the distributions to be normal. Fig. 3, which shows the deviations for

bands 2 and 11 for "a", is an example. The mean square deviation is 4 dB in both cases.

Comparison of the dispersions (S_i^2) for the various bands of a phone shows that the differences are so small that they can be considered as random representatives of the same general dispersion (see Hald [25], Romanovskii [26], for criteria of significance in differences in the S_i^2). Each phone can be represented by one quantity

$$\sigma^2 \approx S^2 = \frac{\sum_{i-1}^{k} SSD_i,}{k\,(n-1)}$$

where k is the number of bands, n the number of announcers (particular variants of a phone), SSD_i is the sum of the squares of the deviations for the ith band.

The following values were obtained for "a", "o" and "u": $\sigma_a = 4{\cdot}1$, $\sigma_o = 4{\cdot}0$, $\sigma_u = 3{\cdot}8$. The differences are so small that for future use we assume $\sigma = 4{\cdot}0$ for all without incurring much error.

It is of interest to see whether the deviations from the means in the various bands are independent. Since the greatest correlation is to be expected between adjacent

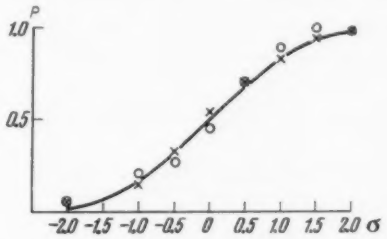

FIG. 3. Distribution of deviations about the mean envelope for "a" in bands 2 and 11.

bands the correlation coefficients were calculated for each adjacent pair. For each phone 10 correlation coefficients (r_i) were thus obtained. Their absolute values are all small ($<0{\cdot}2$), each assembly of r_i presenting both positive and negative values. Hence we conclude that the deviations are mutually independent (see Romanovskii [26] for criteria of significance for correlation coefficients).

The statistical treatment thus shows that when the spectra are split up into fairly wide bands (200 mel each) the deviations of the envelope from the mean within a band follow a normal law, σ being the same for all bands, the deviations in adjacent bands being unrelated.

We could thus pass to the calculation of the recognizability of some sound from analysis of its spectral envelope.

CALCULATION OF SPECTROGRAM "IDENTIFICATION"

The above calculations gave the mean envelope for any phone (where x_1 = u, x_2 = o, x_3 = a) as some sequence of numbers M_1, M_2 . . . M_{11}.

For "u", "o" and "a" these are:

"u"	27	21	11	8	−1	−3	−8	−12	−13	−14	−15
"o"	16	17	13	10	1	−4	−5	−7	−11	−14	−16
"a"	11	4	10	13	2	2	−2	−8	−7	−10	−15

From each particular sound we obtain some fresh sequence $(y_1, y_2 \ldots y_{11})$ which will of course differ from the mean sequence. The problem then consists in determining what phone this particular sound was or, more accurately, the probability of the sound being a particular phone for each phone (x_1, x_2, x_3) separately. In other words, we have to determine $p_y(x)$—the probability distribution for x with y known.

According to Bayes' theorem

$$p_y(x) = \frac{p(x)\, p_x(y)}{\Sigma\, p(x_j)\, p_{xj}(y)}$$

where $p(x)$ is the *a priori* probability of x (here 1/3, since "u", "o" and "a" are equally probable), $p_y(x)$ is the *a priori* probability of x when y is present and $p_x(y)$ is the probability of finding y produced by the hypothesis x (here the probability of finding a given envelope value when the phone of interest, x_j, is pronounced).

As we have already remarked, our particular sound is represented by a sequence of envelope values for the 11 bands. It is clear that even the first band will give us some idea of the phone as it will alter the *a priori* probabilities $p(x_1)$, $p(x_2)$, $p(x_3)$ into the *a posteriori* probabilities $p_{y_1}(x_1)$, $p_{y_1}(x_2)$, $p_{y_1}(x_3)$. Hence the *a priori* probability for each fresh band (new measurement) will equal the *a posteriori* probability of x for the preceding one.

The *a posteriori* probability of x for the last band $p_{y_{11}}(x)$ will be $kp(x)p_x(y_1)p_x(y_2) \ldots p_x(y_{11})$ (Woodward [27]).

Here we assume x to have only three possible values, x_1, x_2 and x_3. These will have *a posteriori* probabilities:

$$\begin{aligned} p_{y_{11}}(x_1) &= kp(x_1)\, p_{x_1}(y_1)\, p_{x_1}(y_2) \ldots p_{x_1}(y_{11}) \\ p_{y_{11}}(x_2) &= kp(x_2)\, p_{x_2}(y_1)\, p_{x_2}(y_2) \ldots p_{x_2}(y_{11}) \\ p_{y_{11}}(x_3) &= kp(x_3)\, p_{x_3}(y_1)\, p_{x_3}(y_2) \ldots p_{x_3}(y_{11}) \end{aligned}$$

where $p(x)$ is known (1/3) and the common factor k is readily found from the condition that

$$p_{y_{11}}(x_1) + p_{y_{11}}(x_2) + p_{y_{11}}(x_3) = 1.$$

The problem therefore amounts to computing the product $\Pi p_x(y_i)$.

Statistical analysis of the y_i for each x shows that they are normally distributed about their means M_i with $\sigma = 4$ dB.

Hence

$$p_x(y_i) = (\sigma\sqrt{2\pi})^{-1} \exp\,[-(y_i - M_i)^2/2\sigma^2].$$

Thus

$$p_{y_{11}}(x) = kp(x)(\sigma\sqrt{2\pi})^{-1} \exp\,[-(y_1 - M_1)^2/2\sigma^2](\sigma\sqrt{2\pi})^{-1} \exp\,[-(y_2 - M_2)^2/2\sigma^2] \ldots$$
$$\ldots (\sigma\sqrt{2\pi})^{-1} \exp\,[-(y_{11} - M_{11})^2/2\sigma^2].$$

After eliminating $p(x) \cdot (\sigma\sqrt{2\pi})^{-11}$ as a common factor we have

$$p_{y_{11}}(x) = k \exp\,[-\Sigma(y_i - M_i)^2/2\sigma^2].$$

We thus have to compute $t = \Sigma(y_i - M_i)^2/2\sigma^2$, e^{-t} being found from tables.

The $p_{v11}(x)$ for several individual spectrograms were computed by this method. We give below the results for spectrograms which it was completely impossible to identify from the frequency of the strongest component.

Spectrogram of an "a" group sound, strongest frequency f = 600 c/s.
Band values:

12, 8, 17, 15, 4, 3—3, —2, —12, 11, —11, —11, —18

	x_1 = u	x_2 = o	x_3 = a
$\Sigma(y_i - M_i)^2$	518	243	117
t	16·2	7·4	3·5
$t^{(t-3)}$	—	0·0122	0·6065
$p_{y_{11}}(x)$	—	0·02	0·98

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Spectrogram of an "a" group sound, strongest frequency f = 600 c/s.
Band values:

10, 5, 16, 13, 6, —1, —6, —7, —10, —12, —17

	x_1 = u	x_2 = o	x_3 = a
$\Sigma(y_i - M_i)^2$	66·7	242	94
t	20·4	7·5	2·9
$e^{-(t-2)}$	—	0·0038	0·3945
$p_{y_{11}}(x)$	—	0·01	0·99

Spectrogram of an "o" group sound, f = 900 c/s.
Band values:

20, 10, 11, 12, 2, —2, —7, —2, —11, —18, —17

	x_1 = u	x_2 = o	x_3 = a
$\Sigma(y_i - M_i)^2$	321	124	280
t	10	3·8	8·7
e^{-t}	0·00005	0·02237	0·00017
$p_{y_{11}}(x)$	—	0·99	0·01

Spectrogram of an "o" group sound, f = 300 c/s.
Band values:

19, 18, 17, 10, 4, —4, —5, —9, —9, —21, —21

	x_1 = u	x_2 = o	x_3 = a
$\Sigma(y_i - M_i)^2$	306	117	537
t	9·5	3·6	16·7
e^{-t}	0·00007	0·02732	—
$p_{y_{11}}(x)$	0·01	0·99	—

Spectrogram of a "u" group sound, f = 375 c/s.
Band values:

26, 28, 11, 13, 2, —4, —10, —14, —15, —16, —17

	x_1 = u	x_2 = o	x_3 = a
$\Sigma(y_i - M_i)^2$	105	330	1042
t	3·3	10·3	—
e^{-t}	0·03688	0·00005	—
$p_{y_{11}}(x)$	0·99	0·01	—

These examples show that our method enables one to determine (differentiate) sounds when no analysis based on the strongest frequency is possible.

THE FRACTION OF SOUNDS WHICH CAN BE DISTINGUISHED BY ENVELOPE METHODS

To judge the value of this method it is clearly insufficient to give a few examples of its successful use. The fraction (per cent) of sounds which can be correctly recognized by its use is a quantitative index of its adequacy. As regards our circumstance we may say that the method is the more reliable the greater the probability that on selecting some arbitrary specimen from the assembly of values of x_1 we get $p_y(x_1) > p_y(x_2)$ and $p_y(x_1) > p_y(x_3)$.*

So we must investigate $P^{\mathrm{I}}[p_y(x_1) > p_y(x_2)]$ and $P^{\mathrm{I}}[p_y(x_1) > p_y(x_3)]$ for group "u" sounds (series x_1), $P^{\mathrm{II}}[p_y(x_2) > p_y(x_1)]$ and $P^{\mathrm{II}}[p_y(x_2) > p_y(x_3)]$ for group "o" sounds, etc.

Since $p_y(x_1) = k \exp[-\Sigma(y_i - M_{i1})^2/2\sigma^2]$ and $p_y(x_2) = k \exp[-\Sigma(y_i - M_{i2})^2/2\sigma^2]$ we can replace the condition $p_y(x_1) > p_y(x_2)$ by $\Sigma(y_i - M_{i1})^2 < \Sigma(y_i - M_{i2})^2$.

For x_1 group sounds $y_i - M_{i1} = \varepsilon$ (deviation from the mean) and $M_{i2} = M_{i1} + l_i$, where l_i is the difference between the mean values of x_1 and x_2 in that particular band.

Thus, we may rewrite our condition as

$$\Sigma\varepsilon_i^2 < \Sigma(\varepsilon_i - l_i)^2 \text{ or}$$
$$\Sigma\varepsilon_i^2 < \Sigma\varepsilon_i^2 - \Sigma 2 l_i \varepsilon_i + \Sigma l_i^2$$
$$\Sigma l_i \varepsilon_i < \frac{1}{2}\Sigma l_i^2.$$

Since ε is a random quantity normally distributed with parameters $m = 0$ and $\sigma = 4$ dB and the l_i are constants, we can also deal with the random quantity $\Sigma l_i \varepsilon_i$. Its mean is zero, since for all $l_i \varepsilon_i$, $m = 0$. Also, $\sigma \Sigma l_i \varepsilon_i$ is easily determined since it has been shown by experiment that σ is the same for all bands. Hence $\sigma l_i \varepsilon_i = l_i \sigma$ and $\sigma \Sigma l_i \varepsilon_i = \sigma \sqrt{\Sigma l_i^2}$, in accordance with the rule for combining dispersions.

We can now determine

$$p(\Sigma l_i \varepsilon_i < \frac{1}{2}\Sigma l_i^2).$$

* Even with an ideal method we would not always get $p_y(x_1) > p_y(x_2)$ for every x_1 group spectrogram. Thus, Peterson and Barney [28] have shown that vowel sounds are far from always (by all speakers) pronounced so clearly that they are correctly recognized by ear. Morever, the very process of recording a spectrum involves certain errors which may arise from the peculiarities of the analyser or from inaccuracies in the choice of region to be analysed.

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TABLE 1. FORMANT COMPOSITIONS OF RUSSIAN VOWELS c/s*

Vowel	Tomson [12]	Borogoditskii [13]	Samoilov [14]	Shcherba [15]	Kazanskii and Rzhevkin [16]	Miasnikov [17, 18]	Varshavskii and Litvak [6]	
							Synthesis	Frequency restrictions
u	682	128	128 512–640	<212 682–768	259	550–850 1750–2050 2650–2950	200–600	Below 1400 (M) 1800 (F)
o	800–900	320	240–532	710 1920–2560	517 1035	550–850	400–800	600–1600 (M) 550–1600 (F)
a	1280–1536	192	768–852	768 960 2304–2844	650 1180	250–1150 2650–2950	1000–1400	650–1900
e	1600–1776	212	240–532 960–2132	<1776	—	550–850 1750–2050 2650–2950	600–1000 1600–2500	800–2800 (M) 850–3350 (F)
i	2132–2400	240	160–192 640–768 2304–2560	1280–1920 2844	453 3000	250–550 1150–1450 2050–2350 2650–2950	2800–4200	1200 and above

* Tomson's and Borogoditskii's data were obtained by aural frequency determination on spoken vowels. Samoilov's, Shcherba's, Kazanskii's and Rzhevkin's data were obtained by harmonic analysis of oscillograms. Miasnikov's data were obtained with band filters. The table gives the bands of the filters showing the largest outputs. Varshavskii and Litvak's data were obtained in recognition studies on synthetic sounds and on natural sounds subjected to frequency restrictions.

This is

$$\frac{1}{2\pi}\int_{-\infty}^{z} \exp(-t^2/2)\,dt$$

where

$$z = \Sigma l_i^2/2\sigma\sqrt{\Sigma l_i^2} = \sqrt{\Sigma l_i^2}/2\sigma.$$

We can thus determine the probabilities in which we are interested by computing z and using the tables for the probability integral.

The following data were thus obtained:

Group "u" sounds:

$$P^{I}[p_y(x_1) > p_y(x_2)] = 0{\cdot}95$$
$$P^{I}[p_y(x_1) > p_y(x_3)] = 0{\cdot}99$$

Group "o" sounds:

$$P^{II}[p_y(x_2) > p_y(x_1)] = 0{\cdot}95$$
$$P^{II}[p_y(x_2) > p_y(x_3)] = 0{\cdot}98$$

Group "a" sounds:

$$P^{III}[p_y(x_3) > p_y(x_1)] = 0{\cdot}99$$
$$P^{III}[p_y(x_3) > p_y(x_2)] = 0{\cdot}98^{*}.$$

These data show that the probability that a sound which is a variant of some phone will be determined correctly is very great, although not unity. This is due to the residual lack of definition—either in the original data or arising from the losses during treatment inevitable in using wide bands—though to which it is due remains unclear. A final answer evidently demands much statistical material and the comparison of results obtained by dividing up the spectra into bands of different widths.

But it is quite clear that although the form used is approximate the general shapes of the envelopes give much more information on the sound than do estimates based on some of the strongest frequencies only (formants).

The use of spectral envelopes is also adequate as regards aural recognition. Results on the perception of complex sounds show that the timbre of a sound is determined by the loudness distribution and that to predict timbres the loudness levels as well as the frequencies of the components have to be allowed for [29] (Chistovich†). Experiments with synthetic vowels [13] confirm this: they show that the ease of recognition varies regularly with the intensity ratios of the formants in the sound.

The above analysis of spectrograms could of course be performed automatically in a special differentiating device. The above calculations show that such a device should most certainly be built.

CONCLUSIONS

(1) A definite problem in analysing the spectrograms of individual speech sounds is to find the probabilities of the sound representing various phones.

(2) A method of determining these probabilities based on comparing the individual spectrum with a collection of mean standard envelopes is presented. The method

* If σ is taken as the same for all groups, $P^{I}[p_y(x_1) > p_y(x_2)] = P^{II}[p_y(x_2) > p_y(x_1)]$.

† Paper at the Conference on Speech Perception, Leningrad, 1956.

involves considering the individual spectrograms as the product of two factors: the character of the phone and the random peculiarities in pronunciation of the particular speaker.

(3) Application of the method to the spectrograms of three Russian vowel sounds shows it to have advantages over the "formant" method often used.

Translated by J. E. S. BRADLEY

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CHANGES IN DEGREE OF SYLLABLE ARTICULATION WHEN TABULAR MATTER IS READ OUT REPEATEDLY UNDER NOISY AND SILENT CONDITIONS*

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(*Received* 27 *July* 1956)

THE perception of voice signals depends on the adaptability of the hearer and on the reception conditions. When special voice-distorting devices (filters) are inserted in the line, the rise in correct reception of syllables, words, etc. from 80 to 100 per cent can be followed readily [1].

The noise produced by the powerful machinery now used industrially is so intense that it is difficult to converse even with the best telephone apparatus. It is thus of practical importance to study training in voice signal reception under very noisy conditions. It is also of some theoretical value to study auditory adaptation to unfavourable conditions from the point of view of the mechanisms and laws involved in perceiving clear speech.

Topics related to adaptation in articulation have not been much considered. Collard [2] was the first to remark on the need for training and the methods required. Such training is also touched on by the International Advisory Committee on Long-Distance Telephony (IACLDT) [3] and by Bykov [4, 5]. Fletcher and Galt [6] have considered this in more detail. The Budenny Academy of Communications has dealt with this in its monographs [1] and in another of its publications (*Trudy Akademii* for 1951 and 1953), this topic has been discussed very fully. But in most cases adaptation under relatively noise-free conditions is considered.

Here we consider adaptation to conditions involving loud noises of defined spectral composition, as determined by experiment.

METHODS AND APPARATUS

It was proposed to study the perception of speech and sound signals acting on the ear, at the same time as noise, in subjects trained to receive articulation tables to various extents. It seemed logical to feed the noise and useful signals (tones and speech) through the same sound radiator simultaneously.

The work had to be spread over a considerable period (more than a year) so the physical parameters of the broadcast speech should not be less stable than those of the noise and sound signals. In other words, all the subjects, who did not participate simultaneously in the tests, should receive articulation tests exactly alike in loudness and other physical parameters. If these requirements are fulfilled experiments carried out over long periods should give quite comparable results.

The first requirement, i.e. a common sound radiator, was fulfilled by the apparatus constructed by a team in our laboratory (Udalov, Babkin *et al.*). The second—

* Biofizika **2**: No. 4, 452–460, 1957 [Reprint Order No. B10 56].

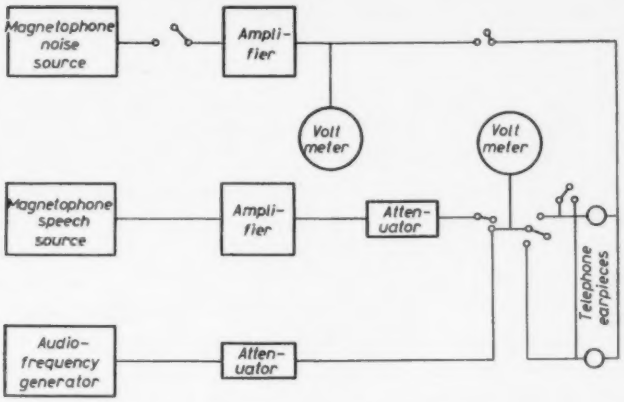

FIG. 1. Block diagram of apparatus.

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identity of spoken material received—was ensured by using magnetophone recordings of tables and a so-called "continuous text". This method is widely used in speech audiometry and was recently recommended by Carchart [7] and Liden [8].

Fig. 1 shows the block diagram of the apparatus used. The ZG-10 audio-frequency generator produced the sound signals. The intensity was adjusted with the attenuator in the instrument, in steps of 1 dB.

The speech signal source was a MAG-5 magnetophone. The mean speech level was adjusted with a special attenuator so the net speech level also varied in 1 dB steps. The same trained announcer recorded all the articulation tables and other speech signals on the tape. The recordings were made at a constant intensity level. The transfer and radiation system did not have a flat frequency response over the whole speech-frequency range. Fig. 2 shows that at low frequencies (100–200 c/s) and at high frequencies (particularly about 2000 c/s) the response was less than at 400–1000 c/s. Hence this circuit introduced considerable speech distortion, so adaptation could be more clearly followed than with a perfect circuit.

The noise, previously recorded on a tape loop from a special noise generator, was generated by a MAG-8 magnetophone system. The noise level was adjusted by

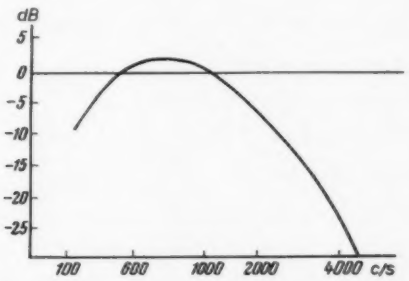

FIG. 2. Frequency response of the speech-signals branch of the apparatus.

altering the output voltage from the two-channel buffer amplifier KTU-100, here used as an amplifier. When the over-all level was altered the noise spectral composition remained sufficiently constant. The noise spectrum was flat from 100 to 5000 c/s. The second channel in the amplifier was used to amplify the speech, when necessary. The noise and useful signal levels were continually checked with a voltmeter which could be switched into various channels.

All three branches of this system could be connected simultaneously or individually to TD-5 dynamic type earphones, using a special switching unit. Two earphones were used so that the work could be speeded up when single-ear reception was used, by employing two subjects.

External disturbances were reduced by placing the subjects in a special room. The whole apparatus was controlled by the experimenter, who was outside the room; he could communicate with the subjects by light signals. The behaviour of the subjects could be observed through a window in the wall of the room.

We used the syllable articulation tables developed in the Institute of Physiology at Leningrad University, in conjunction with workers in the telephony department at the Budenny Academy.

To establish the thresholds for reception and clarity we used texts from newspaper articles (a so-called "continuous text"), spoken by the announcer into a microphone in a quiet even voice without observing pauses due to punctuation. The tables and "continuous texts" were presented in turn to all subjects.

PERSONNEL

We used young men 19–25 years old as subjects, this being approximately the age range suggested by the IACLDT. They were all students at institutes of higher education in Moscow. Preliminary medical examinations revealed no hearing defects. Altogether, 26 subjects were used. Of these, 14 were trained in this experiment to receive speech signals (adaptations experiment), 3 had previously participated in speech articulation tests (with noise present) in our laboratory, 9 had participated in articulation tests not more than three times (in all). Altogether, 203 tests were done (two subjects per test).

The adaptation experiments were begun in June 1954. The subjects experienced various noise conditions from relative quiet to high-intensity white noise. They were required to receive and record various articulation tables. The subjects to be specially trained were all covered by degrees. In the spring of 1955 we had at our disposal the following three groups of subjects, of varying degrees of adaptation:

Group I: 7 well-adapted subjects, who had either participated in the tests since 1953 or had been given special preparation;

Group II: 10 subjects trained for a lesser period (not more than 15 tests);

Group III: 9 untrained subjects.

With these subjects we could perform cross-over tests (of four experiments on any subject two would be in relative quiet and two under noisy conditions). The cross-over experiments were directed to determining the quality of reception for subjects with differing degrees of adaptation.

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The tests (both final and preparatory) were carried out as follows: the subjects (in two's) in the room were given the sound and speech signals either on a noise background or in relative silence. When auditory and speech thresholds were under measurement the signal strengths were increased by the experimenter and the subject gave a lamp signal when the perception level was reached, the attenuator readings being then taken. The measurements were repeated 4–6 times for each threshold and the commonest value taken as the threshold one. These attenuator data were then used in the calculations on auditory acuity. A sound pressure of 2×10^{-4} b was taken as reference level.

The auditory threshold was measured using tones of frequencies 200, 800, 2000 and 4500 c/s. This gave an indication of the subject's hearing over the speech-frequency range. Having found the auditory sensitivity for a subject we proceeded to determine the speech perception thresholds. For this purpose the "continuous texts" were used.

There are several of these so-called speech thresholds. We were concerned with the speech perception threshold, i.e. the intensity at which the speech appeared as a completely unintelligible whisper, and the speech discrimination threshold, i.e. where the separate words could be distinguished but the senses of phrases could not be established.

On passing from the "continuous text" to the articulation tables we increased the loudness by 15 dB over the experimental discrimination threshold. This, in our experience, is adequate to give reproducible results.

The subjects recorded the syllables they heard when listening to the articulation tables and at the end compared their results with the tables, under the control of the experimenter. This check undoubtedly facilitated their adaptation.

RESULTS AND DISCUSSION

Fig. 3 compares the auditory sensitivity of one subject (Vil.) at the start (May 1954) and at the end (April 1955). Two audiograms taken in relative silence are shown. The auditory sensitivity was clearly constant throughout the experiments. The figure also shows (upper curves) that the capacity to perceive sound signals on a

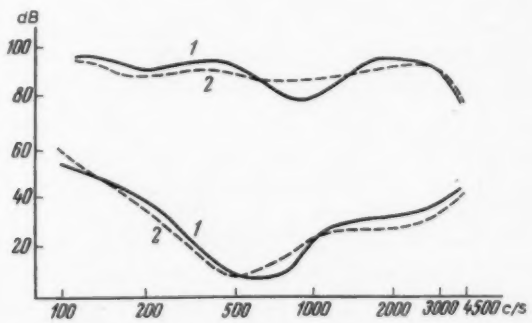

FIG. 3. Auditory thresholds (pure-tone) for subject Vil. in quiet and noisy conditions (lower and upper curves respectively). 1: at start; 2: at end.

noise background was not materially altered. Analogous results were obtained with the other subjects.

This was not the case with the speech perception and discrimination thresholds. In Vil. the former rose by 2 dB during the experiments, and the latter by 1 dB. In Likh. the thresholds were almost constant, in Gog. they dropped by 1 dB, etc. In no case throughout the whole adaptation series did the speech perception threshold shift by more than 5 dB. The capacity to hear sound and speech signals is not essentially altered by experience. This agrees with Tumarkina's data [9].

In our experiments the percentage change of correct syllable reception using repeated large articulation tables was studied. The other forms of articulation, which are mathematically related to syllable articulation scores, are not considered here.

Fig. 4 shows the correct syllable reception during repeated use of the tables for Vil. (1) and Likh. (2) on prior training in relative silence (14 experiments) and then in noisy conditions (15 experiments), and for Gog. (3) who heard the syllable tables once under silent conditions, analogous ones being then used 15 times in succession on a noise background. At the end of the adaptation series the tables were repeated in relative silence. The percentage correctly heard (S per cent) is plotted against

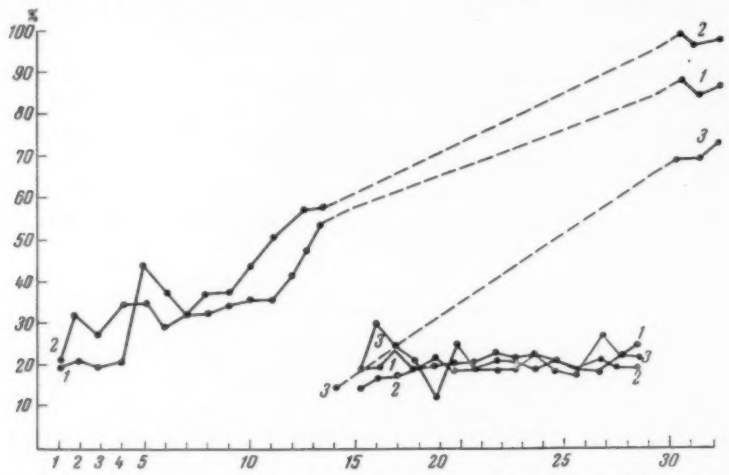

FIG. 4. Effect of training on perception accuracy. 1: subject Vil; 2: Likh.; 3: Gog. Ordinate: percentage correct; abscissa: number of experiment (in sequence). Explanation in text.

number of experiments. In experiments 1–14 Likh. and Vil. showed a gradual uneven rise from about 20 per cent to 55–60 per cent in quiet conditions. Meanwhile Gog., who had heard the tables only in quiet conditions, recognized only 14 per cent in one experiment (No. 14). In experiments No. 15 and onwards, noisy conditions were used. We could find no increase in S: in the 15 tests used the figure was about 20–30 per cent. In Nos. 30–32 quiet conditions were again used. Here Vil. gave 80–85 per cent, Likh. 90 per cent and Gog. 65 per cent. The other subjects gave data comparable with those of Fig. 4, so the results were averaged, the mean data being given in Fig. 5 for all 17 subjects trained on articulation tables. The end section of 1 relates to silence and the start (point 1 on the abscissa) relates to the

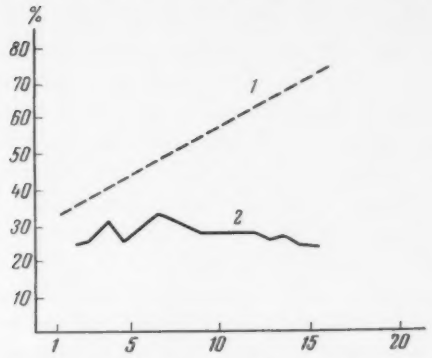

FIG. 5. Effect of training on perception accuracy (average data for 17 subjects). Ordinate: percentage correct; abscissa: number of experiment (in sequence). 1: quiet; 2: noise. Explanation in text.

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mean value in silence in the test immediately before noisy conditions were used. Position 17 on the abscissa similarly relates to the first after the end of the noise experiments. The full line relates to the noise condition experiments.

Fig. 6 gives the mean values found in the final experiments for subjects with differing degrees of training. The figures on the abscissa denote the various groups: I, untrained (9 subjects); II, subjects participating in not more than 15 tests (10); and III, subjects given prolonged preparation (40 or more experiments; 7 subjects). The ordinate gives *S* in per cent.

TABLE 1. PERCENTAGES OF SYLLABLES HEARD CORRECTLY AT VARIOUS STAGES OF TRAINING (MEAN VALUES)

Condition	Untrained	Average training	Well trained
Silence	26	58	65
Noise	28	35	38

Line 1 joins points for quiet conditions, line 2 for noise. The same data are given in Table 1.

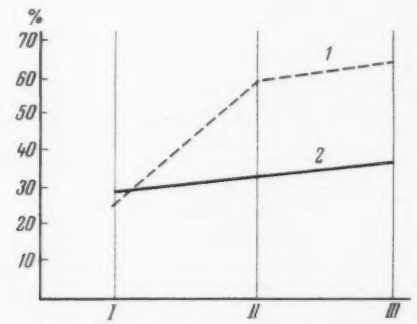

FIG. 6. Mean accuracy of perception for subjects of various degrees of training. Ordinate: percentage correct; abscissa: group of subjects. Explanation in text.

DISCUSSION

Figs. 4 and 5 show that repetition effected no improvement in the experiments where a noise background was used. The rise in quiet conditions is clear.

Comparison of the data obtained in silence before and after the noise series shows that although no improvement occurred during the noise series, the rise in the silence performance was considerable (from 30–80 per cent). So the accuracy rises steadily during training as regards perception in relative silence, no matter whether silent or noisy conditions are used for training. When noise is present the effect of training is masked and not accessible to direct observation.

Passing from these results, which reflect, as it were, syllable perception training dynamics, to the data of Fig. 6, we find that the silence and noise syllable perception results are identical in untrained subjects. Average trained subjects hear 58 per cent correctly in relatively silent conditions (65 per cent for well-trained). In noisy conditions, even the well-trained ones give only 38 per cent. Thus, the difference between untrained and well-trained subjects is 39 per cent in relative quiet and 10 per cent in noisy conditions.

Comparison shows that there is some increase in correct perception in noisy conditions between untrained and well-trained subjects. The rise is very slight relative to that in quiet conditions and it is difficult to consider it as a regular improvement. On the other hand, prolonged training under varying acoustic conditions has no real effect on the auditory acuity for either sound or speech signals. So the cause of the improvement is some process independent of the state of the auditory analyser. At given auditory and speech discrimination thresholds the correct perception of Russian syllables is improved by training. This is seen in the data for the various subjects, where both thresholds vary: the variations have no real effect on the articulation scores.

CONCLUSIONS

The data give rise to the following conclusions.

(1) The auditory acuity and speech discrimination thresholds for silence and loud noise conditions are not affected by repeating the experiments.

(2) When the tables are repeated under noisy conditions there is no clearly developed improvement in performance.

(3) When the tables are repeated under quiet conditions there is a clearly developed improvement in performance, no matter whether training was in quiet or noisy conditions.

Training in perception of speech signals occurs in quiet or noisy conditions, but the results of training appear only when quiet conditions are used for the test.

Translated by J. E. S. BRADLEY

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THE CHARACTER OF SOME HYDROGEN BONDS IN COLLAGEN*

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VOL. 2 1957

(*Received* 1 *February* 1957)

GUSTAVSON's data [1] show that the hydrogen bonds linking the hydroxyproline OH groups to CO groups oxygen play a large part in stabilizing the structure of collagen. But Gustavson's data do not show whether these bonds are inter- or intra-molecular because collagenous tissue specimens were used, in which the flow temperature might be determined either by inter- or intra-molecular polymerization bonds. The bond distribution is also important in determining the chemical and structural properties of collagen particles. Moreover the hydroxyproline and CO group positions in the collagen polypeptide chain are such that the choice of possible structures is restricted.

To elucidate the role of OH . . . OC bonds, the temperature threshold of denaturation must be determined as a function of hydroxyproline content in systems in which there are no important intermolecular interactions. Thus, we may use collagen-like protein solutions, which are [2, 3] sufficiently dispersed and the interactions between the units small. If the bonds are intermolecular there should be no relation between denaturation (flow) temperature and hydroxyproline content in the solutions. If these bonds link intramolecular groups such a relation should be found, and in general it should resemble what Gustavson found for collagenous tissues.

This paper deals with this relation for the collagen-like proteins prepared from calf, pike and cod skin, as Gustavson used.

The soluble collagen-like proteins were prepared by a method developed in Orekhovich's laboratory [2, 4] for preparing procollagen from rat skin. Pieces of skin were cut up with scissors and extracted with citrate buffer at pH 3·8. The proteins were precipitated from the extracts by dialysis against tap water. The deposits were washed with tap water and centrifuged off, and then stored as a moist paste in a refrigerator at 3–5°C.

The thermal denaturation thresholds were measured by preparing solutions with citrate buffer at pH 3·8. The solutions were filtered through sterile filters and used for viscosity measurements, an Ostwald viscometer placed in a thermostatic water-bath being used. The bath temperature was raised from 6 to 45°C at 1–2°C per minute. The denaturation temperature was determined as the inflexion on the

* Biofizika **2:** No. 4, 461–464, 1957 [Reprint Order No. BIO 57].

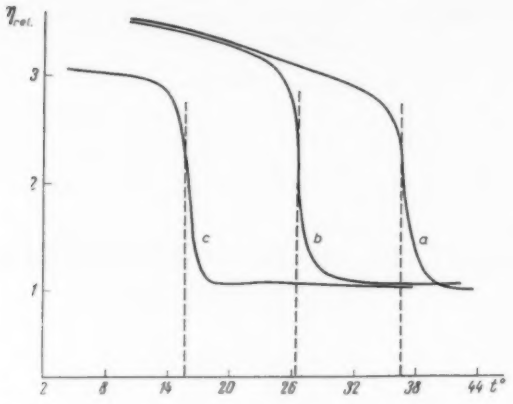

FIG. 1. Relative viscosity for collagen-like proteins vs. temperature: (a) calf skin; (b) pike skin; (c) cod skin.

relative viscosity–temperature curve. This method uses the sharp drop in viscosity at the flow point [5, 6].

The results are shown in Fig. 1. The denaturation temperatures are 37°C for calf skin, 27°C for cod skin and 16°C for pike skin. The data show that the temperature depends on the hydroxyproline content, which is 13, 7 and 5 per cent respectively. This relation is similar to that found by Gustavson for pieces of skin from the same sources. This is implied by the slopes of the denaturation temperature vs. hydroxyproline content curves for our collagen-like proteins and Gustavson's tissues being about the same (Fig. 2).

Additional experiments on calf-skin extract showed that the flow temperature determined in this way was concentration-independent (Fig. 3).

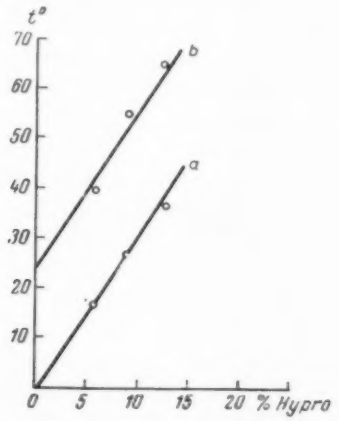

FIG. 2. Thermal denaturation temperature vs. hydroxyproline content for collagen-like proteins: (a) our data on solutions; (b) Gustavson's for collagenous tissue.

The relations found, and the identity between our curve and Gustavson's, indicate that the hydroxyproline OH-group hydrogen bonds are intramolecular. The higher flow temperature for the tissue, compared with that of the protein solution, indicates that energy has to be supplied to break polymerization bonds in the tissue case, but that these bonds do not determine the inherent stability of the internal molecular structure.

The concentration independence of the denaturation threshold indicates that there is no important degree of aggregation in solution. In this connexion it is of interest to pause over certain aspects of collagen structure models. The current conception is that the structural units in collagen consist of three chains forming helices on a common cylinder. Each chain, according to Rich and Crick [7] and Cowan *et al.* [8], has a structure similar to those of poly-L-proline and polyglycine.

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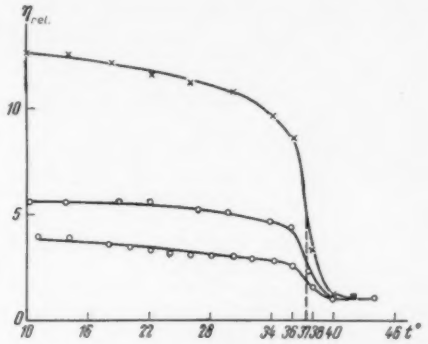

FIG. 3. Solution viscosity vs. protein concentration for calf-skin material. The curves relate to different protein concentrations.

Rich and Crick have shown that only two structural models can correspond to this conception. Both are similar to Ramachandran's model [9] as regards the sub-chain configuration and only allow of one systematic hydrogen-bond network between CO and amide groups. In structure I the NH . . . OC hydrogen bonds are directed counter-clockwise, in II clockwise. In I the hydroxyproline OH-group hydrogen bonds are intermolecular, while in II they also bind the three sub-chains into structural units. Crick supposes both these models to be stereochemically equally satisfactory (although he considers II the more probable), the choice being determined by the direction of the hydroxyproline OH-group hydrogen bond to the CO-group oxygen in relation to the structural unit.

Both of Crick's models can be derived from Ramachandran's by introducing certain modifications. To do this each of his three sub-chains must be turned to right or left through a definite angle. But Ramachadran considers that the model in which the OH . . . OC bonds are intermolecular is more probable, since this agrees better with the X-ray data and gives better atomic packing.

Our data give rise to some comments on the above structural models for collagen. They indicate that Gustavson's bonds are actually intramolecular, i.e. bind chains within the dissolved units. But since the transverse dimensions of these units are, from various physicochemical data, 12–20 Å [2, 3], while the X-ray data on the

hydrated structural units gives transverse dimensions of 17 Å we may consider the units, within the error of experiment, identical. Then the OH . . . OC hydrogen bonds must be directed within the structural units. We therefore consider Crick's model I the more probable as it allows of intramolecular OH . . . OC bonds.

I am deeply indebted to V. O. Shpikter for proposing the present investigation and for guidance in the experimental work.

SUMMARY

The viscosity changes in solutions of collagen proteins derived from calf, pike and cod skins have been studied. Comparison of the thermal denaturation thresholds in solution with the hydroxyproline contents shows that the hydroxyproline OH group–CO group hydrogen bonds lie within the molecules. The structural models for collagen are considered in the light of this data.

Translated by J. E. S. BRADLEY

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A DETERMINATION OF THE MOLECULAR WEIGHT OF INSULIN BY RADIATION METHODS*

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(*Received* 18 *December* 1956)

THE relative fall in biological activity shown by enzymes, hormones, viruses and phages when acted on by various ionizing radiations has already been used to determine particle sizes for these substances [1, 2]. The minimum particle size showing the specific biological properties is often the same as the molecular size, so an independent determination of molecular weight by radiation methods is possible. Heavy particles and fast electrons have mostly been used, X- and γ-rays but little.

The radiation method has naturally to be checked on pure samples of biologically active materials, the data obtained being compared with those derived from classical methods of determining molecular weights. Insulin, a crystalline protein hormone

* Biofizika **2:** No. 4, 465–468, 1957 [Reprint Order No. BIO 58].

which has been much studied, is the most interesting as its molecular weight has been repeatedly determined by various methods (ultra-centrifugation, light-scattering, osmotic pressure, etc.). Edsall [3] concludes that the fundamental unit in insulin in acid aqueous solution has a molecular weight of 12,000 and is in equilibrium with polymers ($M = 24{,}000$, 36,000, etc.). Work on the chemical structure of insulin (Sanger *et al.*) and X-ray data [4] show that the molecular unit in insulin is a particle of $M — 12{,}000$. On the other hand Setlow and Doyle [5] find from the action of fast deuterons and electrons on dry amorphous insulin that $M = 23{,}000$, as if the elementary biologically effective particle inactivated by irradiating the dry material were a dimer, a result difficult to bring into agreement with the chemical and X-ray data. In view of the discrepancy, and to check the radiation method, we studied the radiation inactivation of crystalline insulin (X- and γ-rays) and calculated the molecular weight from the resulting data.

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EXPERIMENTAL

We used a dry crystalline zinc insulin preparation of activity $2{\cdot}1 \times 10^7$ units per mg. The X-ray source was a close-focus BF tube (90 kV, 5 mA, $\lambda = 0{\cdot}6$ Å), dose rate 5000 r/min.* The ^{60}Co γ-ray irradiations were at 5000 r/min.* Evenly spread thin layers of 20–40 mg of dry insulin at the bottom of Perspex cups were irradiated. A flow of cooling water was maintained during irradiations: between irradiation and biological testing the preparations were stored in desiccators at 4°C.

The biological activity was determined from the drop in blood sugar level (in mg per cent) in rabbits after injecting hydrochloric acid solutions of the insulin (10^6 units per kg). The blood sugar was measured (Hagedorn and Jensen's micro-method) at 1·5, 3 and 5 hr after the injection. Rabbits weighing 1·8–2·5 kg were used, initial blood sugar levels being 80–120 mg per cent; 18 hr before the injection the rabbits were weighed and placed in special cages without food. The amount of the irradiated preparation causing the same fall in blood sugar level as a known dose of standard (non-irradiated crystalline) insulin was determined on the same animals, using standard solutions containing 2×10^8 units per ml of 0·0033N HCl (pH 3·5). After preliminary measurements on groups of 3–4 rabbits the final determinations were made using larger numbers (24 in all per experiment). Cross-over experiments were also done, i.e. animals which first received the standard preparation received the irradiated sample after a day, and vice versa. The degree of radiation inactivation was determined from the mean values (using the three blood specimens taken after the injection) for the two groups receiving standard and irradiated material respectively. Tables 1 and 2 give the results, which are also shown in a semilogarithmic plot in Fig. 1.

Fig. 1 shows that the 63 per cent inactivation dose (37 per cent residual activity dose) is $44 \pm 1 \times 10^6$ r (Tables 1 and 2). This dose corresponds [2] to $7{\cdot}05 \times 10^{19}$ ionizations per ml dry protein, and for the types of radiation we used we can assume the ionization uniformly distributed, one ionization having available a volume of $(7{\cdot}05 \times 10^1)^{0-1} = 1{\cdot}42 \times 10^{-20}$ ml. If the inactivation mechanism is a single-hit one (as the linear semilogarithmic plot shows), one ionization at the 37 per cent dose

* We are deeply indebted to V. I. Zatulovskii (Institute of Physical Chemistry, Academy of Sciences of the U.S.S.R.) for his co-operation in the γ-ray experiments.

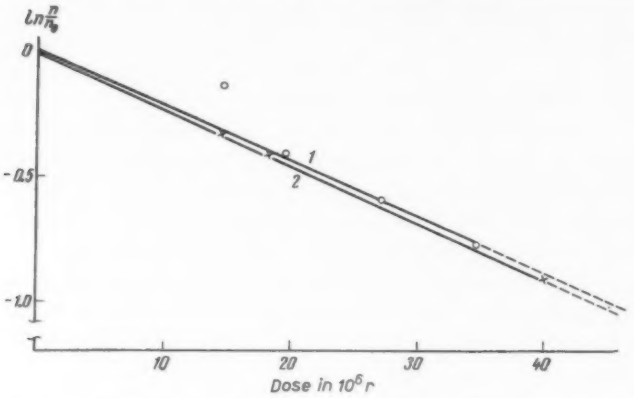

Fig. 1. Residual insulin activity v. radiation dose. 1 = γ-rays; 2 = X-rays.

Table 1. Inactivation of zn-insulin by X-irradiation

Radiation dose (r)	Degree of inactivation (per cent)
14·3	28·0
18·2	34·0
40·0	60·0

may be related to the inactivation of one molecule. Thus, equating the molecular volume to $1{\cdot}42 \times 10^{-20}$ ml the mass (at density 1·3) is $1{\cdot}42 \times 10^{-20} \times 1{\cdot}3 = 1{\cdot}85 \times 10^{-20}$ g, so the molecular weight must be $1{\cdot}85 \times 10^{-20} \times 6 \times 10^{23} = 11{,}100 \pm 300$. As the various types of radiation used differ and there may be errors in the radiation

Table 2. Inactivation of zn-insulin by γ-rays

Radiation dose (r)	Degree of inactivation (per cent)
14·4	(12·8)
19·6	34·0
27·2	45·0
34·7	55·0

dosimetry and in the biological tests, the value obtained may be considered in quite satisfactory agreement with the most reliable values obtained by other methods (12,000 [3, 4]).

Our results also give the size of the elementary biologically active particle as being the same as that of the molecule. The discrepancy in Setlow and Doyle's value [5] can, in our opinion, be explained as due to the differences in the types of preparation and radiation used.

The agreement obtained confirms our original assumption that a single ionization within an insulin molecule inactivates it. The molecular weight of dry lipoxidase (120,000) had previously been determined by Budnitskaia *et al.* [6] on the same assumption, the value being in satisfactory agreement with other data. Thus, at least for molecular weights of 10^4–10^5, the values can be determined from the X- or γ-ray inactivation of biologically active substances. A considerable advantage of this method is that the substances do not have to be separated in a chemically pure form, which is frequently of great value in biological work.

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SUMMARY

The molecular weight of the biologically active particles in crystalline insulin has been determined by X- and γ-ray inactivation to be 11,000 $\pm$ 300.

Translated by J. E. S. Bradley

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THE ACTION OF SMALL DOSES OF IONIZING RADIATION ON DEOXYRIBOSE NUCLEOPROTEINS*

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(*Received* 17 *November* 1956)

The effects of low (up to 1000 r) radiation doses on nucleoproteins have been little studied. Only very recently evidence has appeared that on irradiating nucleoproteins (NP) at 50–5000 r the viscosity of the solution falls [1, 2]. Such studies with low radiation doses, close to the lowest doses producing substantial biological effects, on compounds as important to life as NP, are quite clearly of great importance.

* Biofizika **2:** No. 4, 469–475, 1957 [Reprint Order No. BIO 59].

We have studied the effects on the physicochemical properties of DNP (deoxyribose nucleoprotein) of doses up to 1000 r to elucidate the mechanisms of the radiation effects. It was of particular interest to compare the changes in their physico-chemical and enzymatic properties as produced by γ-rays. It has been claimed that DNP has a phosphatase activity [3, 4].

METHODS

We used calf thymus and rat liver DNP preparations. The first preparation was made by Mirsky and Pollister's method [5] as modified by Lakk [6] and Diskina [4]. The DNP preparation was reprecipitated twice.

The rat liver nucleoprotein was prepared by a rapid method. The rats, anaesthetized with ether, were decapitated. After washing out the liver it was removed and homogenized with $2\frac{1}{2}$ times its volume of 0·13 M NaCl + 0·01 M sodium citrate. Subsequent treatment was as above, but the extraction period was reduced to 12 hr.

The phosphatase activity was determined by Diskina's method [4]. The viscosity was measured in an Ostwald viscometer at 25 ± 0·01°C.

Absorption spectra were taken on an SF-4 spectrophotometer from 240 to 300μ. Error of measurement was ± 1 per cent. The DNP concentrations were determined from the micro-Kjeldahl N determinations.

Fibre production and contractile property determination were by Spitkovskii's method [7]. The optimum conditions for relaxation had earlier been established by Diskina [4] and by Golubeva. The relaxation time was 5 min, the fibre thickness about 0·20 mm, 20 filaments being measured and the mean taken in each case. A GUT-400-1 unit was used for the irradiation, with a dose rate at 1 cm of 23,705 r/min. The DNP solutions were irradiated in 4 ml volumes, giving columns 3 cm high in test-tubes. The tubes were placed at 12 cm from the focus, the dose being computed appropriately.

EXPERIMENTAL

The DNP was first irradiated at 1000 and 25,000 r. Tables 1 and 2 give the results.

The doses used produced a reduction in both the phosphatase activity and the viscosity. With a dose of 10,000 r irradiated DNP gave no filaments, with 5000 r the filaments were poor and did not relax, and only with 1000 r and below did they relax. These doses produced an increase in the extinction coefficients at 260μ. The

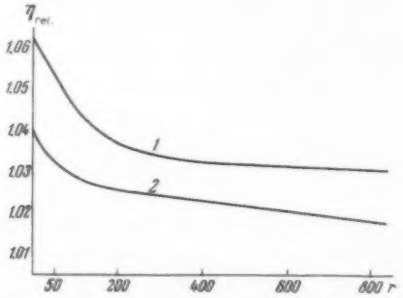

Fig. 1. Relative viscosity vs. radiation dose for DNP solutions. 1—thymus DNP. 2—rat liver DNP.

TABLE 1. CALF THYMUS DNP PHOSPHATASE ACTIVITY AS A FUNCTION OF RADIATION DOSE (μg P per mg N)

Concentration (per cent)	Dose	Phosphatase activity	Per cent inactivation
0·1	0	21·4	0
	1000	13·4	38
	25000	2·6	88
0·2	0	21·4	0
	1000	13·3	45
	25000	5·0	80

TABLE 2. RELATIVE CHANGE IN CALF THYMUS DNP VISCOSITY AS A FUNCTION OF RADIATION DOSE

Dose (r)	η_{irel}	Remarks
0	1·029	0·1 per cent DNP solutions used.
1000	1·023	Viscosities determined on 0·005
250000	1·012	per cent solutions in 1 M NaCl

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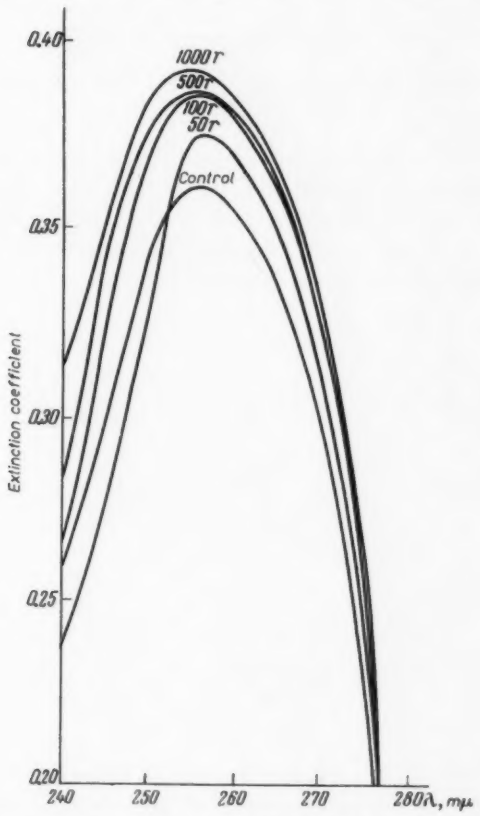

FIG. 2. Absorption spectrum after various doses of radiation to thymus DNP solutions.

data show that even at relatively low doses the DNP changes sharply in properties, the changes being related to depolymerization.

Solutions of DNP were then irradiated with up to 1000 r. Figs. 1–4 summarize the data. The data show that even at low doses the phosphatase activity and physicochemical properties are altered. The drop in viscosity is most rapid around 50–100 r. On increasing to 1000 r the fall continues, but less rapidly. The absorption maximum also increases in height most rapidly up to 100 r. Similar results were obtained with other DNP preparations from thymus and rat liver.

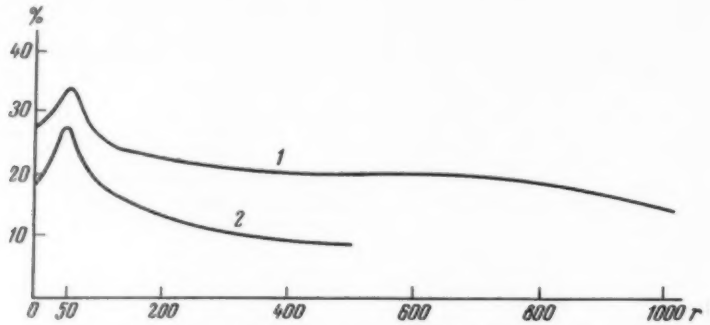

Fig. 3. Relative relaxation vs. radiation dose for DNP. Ordinate: relative relaxation in per cent. Abscissa: dose in r.

A somewhat unexpected result was obtained with DNP filaments: Fig. 3 shows that the elastic properties were accentuated at doses of about 50 r. This rise is not due to experimental error, since the differences are many times greater than the error. Similar results were obtained on the phosphatase activity. In this case (Fig. 4) at 50 r all preparations showed increased activity.

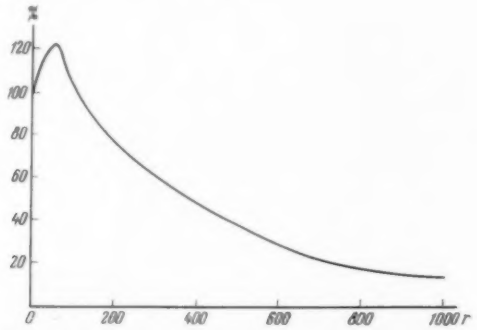

Fig. 4. Thymus DNP phosphatase activity vs. dose. Ordinate: per cent phosphatase activity relative to non-irradiated DNP. Abscissa: dose in r.

DISCUSSION

First of all we consider the rise in relaxation coefficient found with low doses. This cannot be explained in the usual way as due to radiation depolymerization, since the elastic deformation would then be lower. We may perhaps assume that the

DNP molecule becomes less asymmetric at 50 r because of rupture and redistribution of the bonds between the DNA and the protein. This is also responsible for the increased relaxation coefficient. But the extinction coefficient at the absorption maximum meanwhile increases. Particle aggregation, which could produce increased absorption, does not occur here. This is indicated by the reduction in viscosity, as the viscosity would increase with the degree of aggregation. We must therefore suppose that the depolymerization results in increased absorption. Two processes may occur at once on irradiation: some reduction in molecular asymmetry, as the relaxation measurements indicate, together with depolymerization, as the spectrophotometric data would indicate.

We must therefore suppose that at about 50 r depolymerization is slight, the elastic properties not being much affected. The viscosity falls with the asymmetry as with the degree of polymerization. The observed viscosity drop is probably due to the sum of the two effects. The viscosity–radiation dose relation is not linear, unlike the normal result at high radiation doses. At low doses the viscosity drop is relatively larger than at high ones. This partially confirms our view that two processes occur together. At high doses there is practically no change in molecular symmetry, the viscosity change being wholly due to depolymerization, the viscosity–dose relation then being linear.

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Under our conditions disaggregation is excluded as we used very dilute solutions (0·018 per cent). We now turn to how the reduced asymmetry produced by 50 r can increase the reversible deformation.

Spitkovskii [8] has shown that the characteristic viscosity $[\eta]$ of DNA and the mean square distance between the ends of the molecule $\sqrt{\overline{h^2}}$ are related by

$$\log [\eta] = -2{\cdot}5545 + 1{\cdot}14356 \log \sqrt{\overline{h^2}}.$$

We shall assume that this formula is, to an approximation, applicable to nucleoprotein molecules also, since the DNP viscosity is mainly determined by that of the DNA, and the viscosity change after 50 r involves no appreciable molecular destruction. Then the viscosity drop at 50 r corresponds approximately to the change in $\sqrt{\overline{h^2}}$ from 1100 to 1015 Å in one experiment and from 1015 to 938 Å in the second, i.e. 7·6 and 7·5 per cent respectively. The increased relaxation accompanying the decrease in $\sqrt{\overline{h^2}}$ should be 7 and 24 per cent respectively, as direct measurements show. This is in complete accord with published data on enzyme activation by low-dose irradiation [9]. Activation phenomena can be explained as a general rise in energy level and excitation in molecules receiving definite amounts of energy on irradation. We must only note that both activation and deactivation of phosphatase occur in our experiments at doses much lower than for free enzymes. Alkaline phosphatase, for instance, retains its activity at doses of about 10^4 r [10]. We must therefore suppose the nucleoprotein-associated phosphatase to be much more radiosensitive than the free material.

These data confirm an earlier observation [4] on the relation between enzyme and contractile properties in DNP. They also indicate that association with the nucleoprotein makes the enzyme much more radiation-labile. If we consider the protein molecule in DNP surrounded by the DNA then any change in the DNA must to some extent affect the protein. Most probably the DNA depolymerization

occurring above 100 r causes bond redistribution as between DNA and protein, thus producing changes in the protein molecule configuration, which may possibly be of a denaturing type. This, in its turn, would affect the enzyme activity.

Nucleoproteins are, therefore, very radiosensitive. This is partly occasioned by the structure of DNA, which recent data [11] show to have breaks in each of the two spirals about once per 50 nucleotide residues. These breaks are naturally the points where the protein is most vulnerable and, together with other possible factors, may much reduce its resistance.

The nucleoprotein radiosensitivity undoubtedly depends on the extraction method used. The less denatured the DNP after separation, the more labile it will be to external influences, particularly radiation. One reason why we observed changes in physicochemical properties and biological activity in our experiments at relatively low doses may be that the method used provided a relatively "native" preparation.

CONCLUSIONS

(1) It has been shown that DNP solutions given low doses ($\sim$ 50 r) of radiation show physico-chemical and enzymatic changes.

(2) The viscosities of DNP solutions fall on receiving 50 r or more.

(3) The ultra-violet absorption maxima of DNP solutions become higher on receiving 50 r or more.

(4) The contractile properties of filaments prepared from DNP solutions given about 50 r increase the relaxation coefficient falling at higher doses, becoming zero at 1000 r.

(5) The phosphatase activity of DNP solutions given about 50 r increase, the enzyme activity falling at higher doses.

(6) The structural and physicochemical properties of DNP, and the phosphatase activity, are considered to be closely related.

(7) The observed phenomena are considered as due to changes in the DNP molecule produced by the radiation.

Translated by J. E. S. BRADLEY

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DISAGGREGATION BY IONIZING RADIATION IN DEOXYRIBOSE NUCLEOPROTEIN FILAMENTS*

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(*Received* 6 *November* 1956)

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THE processes most readily damaged when an organism is irradiated are those closely related to nucleoprotein metabolism. The damage appears as delay or inhibition of mitosis, chromosome breaks, anomalous cell growth, mutation, sterilization of the reproductive cells, etc. The damage is clearly apparent in organisms after doses of 100–500 r. It is natural to assume that the nucleoproteins are the primary radiation target and that alterations in their states are the causes of the observed morphological and physiological disturbances.

But studies on isolated nucleoproteins and nucleic acids show that gross chemical changes (i.e. liberation of inorganic phosphorus, ammonia, or purine base), or changes in viscosity, flow birefringence, etc., only become apparent at 20,000–50,000 r [1–4]. Although there is not a complete parallel between radiation action *in vivo* and *in vitro*, the large difference in doses suggests that such a parallel cannot hold. It has, of course, been shown by some workers that nucleoproteins and nucleic acids are very radiosensitive. Thus Rozendaal *et al.* [5] state that they could detect the passage of nucleic acid into solution, from the absorption appearing around 260μ, using deoxyribose nucleoprotein (DNP) filaments suspended in physiological saline and doses of 5, 10 and 20 $\times$ 10^3 r. Cole and Ellis [6] have detected changes in DNP after 850 r by trypsin digestion, and Pasynskii [7] has stated that viscosity changes were observed in high-polymer DNP after 50–500 r.

We have previously [8] shown that the radiosensitivity of dissolved DNA can be increased by traces of dissolved trivalent iron, complete loss of structural viscosity being observed after 5000 r. Thus, although the gap between the doses effective *in vivo* and *in vitro* has been much reduced it is still fairly large, so that further attempts to detect high radiosensitivity in dissolved DNP are of some interest.

To this end we studied the radiation disaggregation of DNP filaments suspended in distilled water, the DNP going over to a colloidally soluble state. To increase the sensitivity we made ^{32}P-labelled DNP, filament changes being judged from radioactivity in solution.

The labelled DNP was produced by injecting six rats subcutaneously with Na_2HPO_4 at about 200μ c per rat. At 20 hr after injection, i.e. when the nucleic acid ^{32}P is highest [9], the DNP was extracted from liver and spleen, using an obvious modification of Mirsky and Pollister's method [10]. The tissue was ground up at a low temperature with twice its volume of 0·9 per cent NaCl containing 0·02 M sodium citrate, then washed with one volume of 0·9 per cent NaCl containing 0·01 M sodium citrate, followed by one of physiological saline. The extract was left in the

* Biofizika **2**: No. 4, 476–479, 1957 [Reprint Order No. BIO 60].

refrigerator overnight and then poured off, leaving the deposit. The nucleoproteins were precipitated by pouring the salt extract into 10 volumes of cooled distilled water. The precipitated filaments were removed on a rod and redissolved in 12 volumes of M NaCl containing 1 per cent Na_2HPO_4, to flush out traces of inorganic radioactive phosphorus. The precipitation was repeated three times but without adding more inorganic phosphate. The washing was checked from the water activity after each precipitation and removal of DNP by centrifuging. Table 1 gives these results. It is clear that four precipitations remove inorganic radioactive phosphorus practically completely.

TABLE 1. PREPARATION OF DNP

Precipitation	Activity of 5 ml* of supernatant (counts/min)
First	667
Second	159
Third	47
Fourth	11

* The activities were measured by drying on to metal dishes 18 mm in diameter and 1 mm high and counting at 7 mm from the Geiger–Müller counter.

The purified DNP was dissolved in M NaCl and the concentration determined from the diphenylamine reaction on the DNA. It was found that 10 ml contained 1 mg DNA. The activity was about 0·015μ c per mg DNA. Filaments for irradiation were prepared from this solution. The specimens were prepared as follows: 10 ml portions of the salt solutions were poured with stirring into 100 ml of distilled water. The DNP was then precipitated as well-formed white filaments. When the filaments had settled to the bottom, the water was decanted and the filaments washed with 10 ml of water.

The suspended freely floating filaments were irradiated with X-rays. In most cases two specimens were used with each dose. The irradiation conditions were 180 kV, 10 mA, cardboard filter, focal distances 20 cm (1000, 2500, 5000 and 10,000 r)

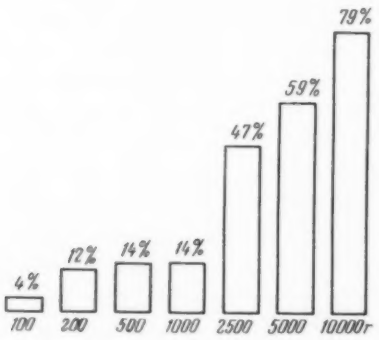

FIG. 1. Passage of radioactive DNP components into a soluble state on irradiation.

TABLE 2. SOLUBLE DNP AFTER DOSES OF X-IRRADIATION, MEASURED AS ACTIVITY OF SUPERNATANT

No of experiment and total activity of specimen (counts/min)	Dose (r)	Total supernatant activity (counts/min)	Mean supernatant activity (counts/min)	Same, as per cent of total activity	Excess over control (in per cent)
1; 4000	Control	415 577	446	11	—
	100	542 605	573	14	3
	200	820 932	876	22	11
	500	1045 985	1015	25	14
	1000	847 830	838	21	10
2; 4700	Control	335 270	300	6	—
	1000	730 1125	925	20	14
	2500	2300 2775	2535	53	47
	5000	3210	3210	68	62
	10,000	4060 4415	4235	90	84
3; 5490	Control	335	335	6	—
	100	607 560	584	11	5
	200	1058 1077	1068	19	13
	1000	1331	1331	24	18
4; 4941	Control	792	792	12	—
	2500	2950 3070	3010	60	48
	5000	3380 3450	3415	69	57
	10,000	4375 4085	4230	86	74

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and 30 cm (100, 200 and 500 r and some of the 1000 r experiments), dose rates 454 and 97 r/min respectively. Control specimens were prepared simultaneously. Both sets were incubated at room temperature for 4 hr, after which the insoluble filaments were removed by centrifuging for 10 min at 3000 rev/min. The supernatant was poured off and two 4 ml specimens taken for activity determination. Knowing the total DNP activity used, the fraction of the total activity in the specimen which had gone into a colloidal-soluble state was computed. Table 2 gives the figures, averaged results being given in the diagram.

The data show that some activity appears in the water on standing for 4 hr, this varying from 6 to 12 per cent of the total activity. This variation is clearly dependent on the preparation conditions, as the DNP was prepared afresh for each series. There is a distinct increase in solution activity even after 100–200 r, and the activity rises regularly with dose. The supernatants gave the DNA diphenylamine reaction. On acidification all the activity was in the precipitate, showing that it remained in DNP (or DNA) macromolecules and was not split off as inorganic phosphorus, only the insoluble DNP being depolymerized and colloidally-soluble DNP (or DNA) being formed.

It was thus shown that biologically effective doses (100–1000 r) can cause appreciable physico-chemical changes in the native DNP molecules *in vitro*. The data lead us to suppose that at 100–1000 r we also get structural changes in nuclear nucleoproteins *in vivo*, which changes may be responsible for morphological and physiological effects.

CONCLUSIONS

(1) A method of preparing ^{32}P-labelled deoxyribose nucleoproteins from rat tissues is described.

(2) When these labelled DNP filaments are X-irradiated (while suspended in water) the filaments depolymerize and some DNP (or DNA) goes over to a colloidally soluble state, in which it is readily detectable from the radioactivity in the solution.

(3) These changes occur at 100–200 r and increase with dose.

(4) These *in vitro* observations of low-dose changes in the physicochemical state of DNP support the idea that one would also get structural changes in nucleoproteins *in vivo*, which changes may be responsible for morphological and physiological effects.

Translated by J. E. S. BRADLEY

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THE EFFECT OF X-IRRADIATION ON THE SYNTHESIS OF PROTEIN IN THE SHOOTS OF RYE*

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(*Received* 20 *December* 1956)

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VERY little is known of the effect of ionizing radiations on protein synthesis in plants. In our study [1] by vacuum infiltration, we showed that when 10 day old rye shoots were irradiated with 20,000 r of X-rays, an increase of protein synthesis occurs during the first hour after irradiation, although a substantial decrease takes place during the next 24 hr. A single work [2] has since appeared on this problem. In it the effect of X-irradiation on the radicles of 2 day old wheat seedlings that was studied. The authors were able to observe an inconsiderable decrease in protein synthesis on irradiating the radicles with 1000 r. However, these changes were only detected 24 hr after irradiation. After considering the high sensitivity of the isotope method, we decided to apply it as an auxiliary aid for studying protein synthesis in irradiated plants.

The investigations were carried out on 10 day old shoots of rye which were cultivated in light in enamel vessels in water. Two days before irradiation the seedlings were deprived of their endosperms and thus their subsequent growth continued in conditions of nitrogen starvation with normal carbohydrate nutrition obtained by photosynthesis [3]. The plants were exposed to X-radiation at doses of 5000 r and 10,000 r (190 kV, 15 mA, cardboard filter), after which the roots of the irradiated and control seedlings were immersed in a solution of ^{14}C-glycine of specific activity 0·06μ c/ml. After the lapse of definite times (4 and 24 hr) the seedlings were taken out, their radicles cut off and the protein extracted from the green part. Before extraction of the protein, the plant material was treated with acetone for 2 or 3 min in order to remove the cell sap which contains a large amount of inert substances and in particular of inorganic (ash) elements, and also to loosen the protein bonds with the other cell components. The protein extraction was carried out with a 30 times (by weight) quantity of 0·2% NaOH saturated with toluene [4]. Extraction was repeated three times, with an hour's digestion each time. The protein was precipitated by an equal volume of 10% trichloracetic acid and repeatedly (20 times) washed with a 1% solution of the same acid. Taking into consideration the data of Pavlovskaia, Volkova and Pasynskii [5] and also that of Kuzin *et al.* [2], in order to remove adsorbed ^{14}C-glycine we redissolved this protein, which had been carefully washed with trichloracetic acid, in 0·2% NaHO containing excess (1·5–2%) unlabelled glycine and let it stand for 2 or 3 hr at room temperature. The protein preparation, reprecipitated by trichloracetic acid, was freed of nucleic acid by boiling with 5% trichloracetic acid for 15 min [6]. Then the protein was washed a few times in 1% trichloracetic acid, alcohol and ether. The protein preparation thus

* Biofizika **2**: No. 4, 480–482, 1957 [Reprint Order No. BIO 61].

obtained was dissolved in 2% KOH and 0·2 ml of the solution placed on a disk for determination of the radioactivity in an end-window counter. In a similar amount of protein solution the protein concentration was determined by nitrogen analysis. The results of these experiments are given in Tables 1, 2 and 3.

TABLE 1. EFFECT OF 5000 r ON PROTEIN SYNTHESIS IN RYE SHOOTS DURING DIFFERENT PERIODS AFTER IRRADIATION

No. of experiment	Time after irradiation (hr)	No. of counts/min in 10 mg protein		Percentage experimental over control
		Control	Experimental	
1	4	265 ± 5	285 ± 3	108 ± 1·6
2	4	653 ± 10	948 ± 18	145 ± 2·7
3	24	2034 ± 3	2017 ± 21	99 ± 1

TABLE 2. EFFECT OF 10,000 r ON PROTEIN SYNTHESIS IN RYE SHOOTS 4 hr AFTER IRRADIATION

No. of experiment	No. of counts/min in 10 mg protein		Percentage experimental over control
	Control	Experimental	
1	156 ± 3	115 ± 4	75 ± 3·2
2	169 ± 2	145 ± 2	86 ± 1·8

TABLE 3. EFFECT OF 10,000 r ON PROTEIN SYNTHESIS IN RYE SHOOTS 24 hr AFTER IRRADIATION

Time of desorption (hr)	No. of counts/min in 10 mg protein		Percentage experimental over control
	Control	Experimental	
2	1694 ± 25	433 ± 5	25 ± 1
24	1790 ± 31	511 ± 19	28 ± 1

As is clear from Table 1, in the first hours after irradiation with 5000 r an increase of protein synthesis is observed. But during the subsequent hours the rate of protein synthesis falls and in 24 hr reaches the level of the control. On increasing the dose to 10,000 r the changes in protein synthesis have a somewhat different character (Tables 2 and 3).

Thus 4 hr after irradiation an inhibition of protein synthesis was noted, approximately equal to 15–20%, which increased with time and in 24 hr reached 75% (Table 3).

From the data presented it is clear that, in the first hours after irradiating the seedlings with a certain dose increase of protein synthesis can be observed. But the question naturally arises as to whether the observed increase in radioactivity in the protein of irradiated plants is the result of an increased adsorption of labelled glycine

on the surfaces of protein globules, which are changed under the influence of irradiation [2, 5]. Special experiments showed that the method of treatment of the protein preparations which we used in our work excluded such a possibility of removal of radioactive contaminations. Thus, by redissolving in alkali and by allowing to stand for 2 hr with unlabelled glycine, all the adsorbed labelled amino acid was removed; for prolongation of the time of standing to 24 hr did not lead to any additional decrease in the protein radioactivity (Table 3).

It was also necessary to prove that the observed changes in protein synthesis were not due to the radiation affecting the absorption of ^{14}C-glycine by the roots of the seedlings. Such a disturbance would result in a different concentration of this labelled amino acid in the control and the irradiated seedlings. We therefore determined the total radioactivity in irradiated (10,000 r) and control seedlings after keeping them in a labelled glycine solution of specific activity 0·06μ c/ml. These results are presented in Table 4.

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TABLE 4. EFFECT OF 10,000 r* ON THE ABSORPTION OF ^{14}C-GLYCINE BY THE ROOTS OF RYE SEEDLINGS IN DIFFERENT PERIODS AFTER IRRADIATION

Time after irradiation (hr)	No. of counts/min in 100 mg dry weight of shoot		Percentage experimental over control
	Control	Experimental	
4	25555 ± 204	25432 ± 329	99 ± 1·45
24	13412 ± 110	14854 ± 821	110 ± 6·14

*Since protein synthesis was determined in the shoots, it is the shoots for which the dose of radiation is given. Under these conditions the roots received 5000 r.

These facts indicate that, even on irradiating seedlings with our maximum dose, the root permeability was not affected during the first hours after irradiation, while in 24 hr it was somewhat increased (10%). However, this increase in permeability was so small that it could not be substantially reflected in the protein synthesis of the irradiated shoots. Thus, with a particular dose of X-radiation (5000 r) the response of the plant to the radiation is a brief "activation" of protein synthesis. These changes, so it seems to us, have nothing in common with the stimulating effect of radiation, for they are unstable and are observed with comparatively high doses of radiation.

Increase of the dose to 10,000 r leads to an immediate fall in protein synthesis which progresses with time and leads, in 24 hr, to an inhibition of approximately 75%.

CONCLUSIONS

(1) During the first 4 hr after irradiation of 10 day rye seedlings with 5000 r of X-rays an "activation" of protein synthesis is noted. But during the next 24 hr the intensity of protein synthesis falls to the level of the control.

(2) A dose of 10,000 r leads to an immediate fall in protein synthesis by 15–25% This inhibition increases with time and in 24 hr reaches 75%.

(3) The facts presented indicate that the application of the isotope method to the investigation of protein synthesis in an irradiated plant, leads to essentially the same regularities as we observed earlier by using the vacuum infiltration method of investigating protein synthesis. But thanks to the high sensitivity of the isotope method it has been possible to reduce the threshold of the observed radiation action approximately fourfold.

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THE COMBINED ACTION OF ULTRA-VIOLET AND X-RAYS ON THE SPORES OF *BACILLUS ANTHRACIS**

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(*Received* 20 *December* 1956)

IT is well known that ultra-violet rays of about 260μ wavelength, possessing high bactericidal activity, are more effective on vegetative bacterial cells than on bacterial spores. Those doses of ultra-violet radiation which are sufficient fully to inactivate sporeless bacteria, often have little effect on spores. Ionizing radiation also has less effect on spores than on vegetative cells. To be certain of inactivating bacterial spores, a very large dose is usually required—of the order of 2–3 million r [1–3]. Radiobiologists have tried to increase the effect of radiation by combining it with other physical and chemical agents. Thus, for example, Curran and Evans [4] carried out experiments on sensitization of spores to elevated temperatures by means of previous ultra-violet irradiation. Meisel' and Medvedeva [5] investigated the change in sensitivity of yeasts to X-rays under the influence of various factors, among them elevated temperatures. Kempe [6] showed that the bactericidal effect is considerably increased by combining ionizing radiations and high temperature. Similar facts were obtained by Graevskii for β-rays [7]. Aleksandrov [8] analysed, experimentally and theoretically, the nature of the combined action of radiation and temperature. An increase in the biological action of radiations in the presence of various chemical substances has been repeatedly observed [9–13]. Of special scientific and practical interest is the combined action of radiations of different types. By analysis of the

* Biofizika **2:** No. 4, 483–486, 1957 [Reprint Order No. BIO 62].

results of such action one can estimate the similarities and differences in the mechanism of the biological actions of the radiations. Hollaender and his co-workers [14, 15] studied the combined action of infra-red and X-rays on micro-organisms. A vast literature is concerned with the joint action of ultra-violet and visible light leading, in a number of cases, to photoreactivation [16].

When the experimental part of our work was finished, the work of Uretz [17] appeared on the combined action of ultra-violet and X-rays on haploid and diploid cultures of yeast. The author observed that previous ultra-violet irradiation of haploid yeast had only a weak effect on its stability to X-rays. The yeast exposed to X-irradiation behaved as regards ultra-violet, like unirradiated yeast. The diploid cultures reacted differently: in them, in essence, the results of ultra-violet and X-irradiation were additive. On this basis Uretz concluded that both radiations acted at one and the same spot.

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The aim of our work was to study the combined action of ultra-violet and ionizing radiation on the survival of the spores of *B. anthracis* in water. The source of ultra-violet rays was a bactericidal lamp (BUF) with a predominant emission at 2537 Å. For X-irradiation we used a RUP-1 apparatus at 180 kV and 15 mA; when the dose was 100,000 r the dose rate was 920 r/min and when the dose was 50,000 r the dose rate was 1025 r/min. The spores of *B. anthracis*, obtained after heating up old cultures of *B. anthracis* on a water bath for 10 min at 80°C, were irradiated in water by X-rays or ultra-violet rays or exposed to their combined action, after which they were plated on meat–peptone–agar in a Petri dish. After culturing for 24 hr at 35° the number of growing colonies was counted.

In the first experiments on combined action the *B. anthracis* spores were first irradiated with X-rays and then with ultra-violet rays. The experimental results (Tables 1 and 2) showed that, when the actions of both types of radiation are combined, the bactericidal effect is considerably increased—the over-all effect is greater than the sum of the effects of the two factors.

In the next experiments an attempt was made to find out whether it mattered, for the combined action, which of the two radiations acting on the bacterial spores was applied first. With this in view, one part of a sample of the *B. anthracis* spore suspension in water was irradiated first by X-rays then by ultra-violet rays, while another part was irradiated in the reverse order. Parallel samples of the spore suspension were irradiated by ultra-violet rays only and by X-rays only. The experimental results are summarized in Table 3.

TABLE 1. SURVIVAL OF SPORES OF *B. anthracis* ON IRRADIATION IN WATER BY X-RAYS AND ULTRA-VIOLET RAYS AND ON COMBINING THE ACTIONS OF BOTH TYPES OF RADIATION

Radiation and dose	Average number of viable spores in 0·1 ml	Percentage of control
Control	30,900	100
X-rays, dose 100,000 r	5140	16·6
U.V. rays, dose $2{\cdot}31 \times 10^5$ ergs/cm^2	4800	15·5
Combined action: X-rays, dose 100,000 r, and u.v. rays, dose $2{\cdot}31 \times 10^5$ ergs/cm^2	131	0·42

TABLE 2. SURVIVAL OF SPORES OF *B. anthracis* ON IRRADIATION IN WATER BY X-RAYS AND ULTRA-VIOLET RAYS AND ON COMBINING THE ACTIONS OF BOTH TYPES OF RADIATION

Radiation and dose	Average number of viable spores in 0·1 ml	Percentage of control
Control	552	100
X-rays, dose 50,000 r	148	27
U.V. rays, dose $7{\cdot}7 \times 10^4$ ergs/cm^2	273	49
Combined action: X-rays, dose 50,000 r, and u.v. rays, dose $7{\cdot}7 \times 10^4$ ergs/cm^2	17	3·1

TABLE 3. SURVIVAL OF SPORES OF *B. anthracis* ON IRRADIATION IN WATER BY X-RAYS AND ULTRA-VIOLET RAYS AND ON COMBINING THE ACTIONS OF BOTH TYPES OF RADIATION

Radiation and dose	Average number of viable spores in 0·1 ml	Percentage of control
Control	2447	100
X-rays, dose 50,000 r	503	20·5
U.V. rays, dose $7{\cdot}7 \times 10^4$ ergs/cm^2	1425	58·2
Combined action: first X-rays, dose 50,000 r, then u.v. rays, dose $7{\cdot}7 \times 10^4$ ergs/cm^2	206	8·4
Combined action: first u.v. rays, dose $7{\cdot}7 \times 10^4$ ergs/cm^2, then X-rays, dose 50,000 r	192	7·8

The experimental results presented in Table 3 lead to the conclusion that it makes no essential difference to which radiation action the spores are first exposed. The experiments also show that the overall effect of the combined action of the two types of radiation—X-rays and ultra-violet—is considerably greater than the effect of each type separately and even than the sum of the effects of the actions of the same doses of ultra-violet and X-rays. At the same time it does not matter which of these types of radiation—X-rays or ultra-violet—is used to irradiate the bacterial spores first. The facts obtained point to the proximity of the points of action of these two types of radiation. The biological effect of each of these radiations leads not only to irreversible inactivation of the bacterial spores, but also to a different kind of reversible injury which is increased by the additional action of the other type of radiation, leading to lethal inactivation of the spores. It is very probable that the combined action of two or more types of radiation, since this is more effective, can be used for sterilizing water, foodstuffs and medicines.

CONCLUSIONS

By a study of the combined action of ultra-violet ($\lambda = 2537$ Å) and X-rays on the spores of *B. anthracis* it has been shown that the combined effect is considerably greater than the effect of each type of radiation separately, and even than the sum of the effects of the actions of these radiations. At the same time it does not matter which of these radiations—X-rays or ultra-violet rays—is used first to irradiate the bacterial spores.

Translated by J. DAINTY

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TIME DISTRIBUTION OF MORTALITY IN IRRADIATED ANIMALS*

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(*Received* 6 *December* 1956)

The biological actions of ionizing radiations in general, and their actions on mammalian organisms in particular, are extremely complicated. Due to the non-specific absorption of such radiations, to the high energy per single ionization, and to the possibility of secondary interactions, even the initial effects of ionizing radiation on a living substance are quite heterogeneous. Moreover, because of the interrelationships occurring in a living organism, various side effects and concomitant processes are possible. Numerous observations have shown that the death of irradiated animals may, in the final analysis, be due to various causes. One of the problems of radiobiology is the differentiation of these causes and the isolation of individual processes from the complex picture of the radiation syndrome. Such differentiation may be based on different premises. It may be attacked from the biophysical standpoint by way of qualitative analysis of the mortality dynamics in irradiated animals. The present work deals with some results of such analysis, the principles of which were elaborated earlier [1].

* Biofizika **2:** No. 4, 487–494, 1957 [Reprint Order No. BIO 63].

Mice of several strains and white rats were used as experimental animals. The mouse strains used were of different radiosensitivity: $LD_{50/30}$ varying from 410 to 590 r [1]. The animals were exposed to a single whole-body irradiation (using doses of from 200 to 30,000 r) without any additional action. Mice were subjected to γ-radiation from ^{60}Co. Rats were irradiated with X-rays (170 kV, 15 mA, filters 2 mm Al, 0·5 mm Cu). The dose-rate varied from 10 to 290 r/min. The survival times of individual animals were determined within $\pm$ 8 hr. In addition to our experimental results some published findings of other workers have been analysed.

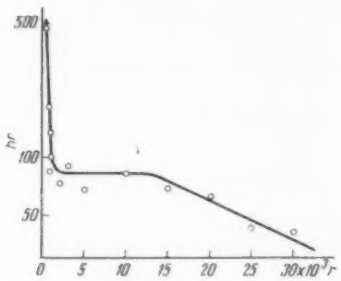

FIG. 1. The dependence of survival time on the dose of γ-rays in "H" strain white mice.

Fig. 1 shows the relationship between the survival of strain "H" mice and the dose of γ-rays. The curve is composed of three sections. At first the time of survival decreases rapidly with increasing dosage; later, with doses of 1000–15,000 r, survival time does not change, being 3·5 days; with still higher doses, there is a further decrease of the mean survival time. Comparable curves have been obtained by other workers [2–5] for X-irradiation. Quastler [2] found that, with doses of the order of $LD_{50/30}$, the mean survival time was most frequently 11·5 days, and he terms these two periods (3·5 and 11·5 days) "the characteristic values of the mouse reaction spectrum". He considers that these "characteristic values" correspond to the latent periods of two distinct mechanisms involved in radiation mortality, one of which plays the major part at low radiation doses and the other at higher ones.

However, it seems doubtful whether the death of irradiated mice is conditioned by two processes only. Furthermore, it may be that with an identical dose the causes of death in different individuals may be different. Thus, it is of interest to compare not only the mean survival time following different dosages, but also the mortality dynamics at a given dose.

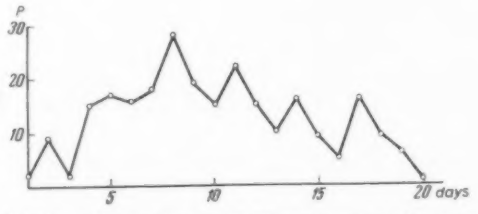

FIG. 2. Time distribution of mortality in "M" strain mice after irradiation with 500 r γ-rays. Ordinate: p, the number of animals which died.

The time distribution of mortality in irradiated mice and rats appears at first glance to be similar to normal. This, however, is not so. This distribution has been statistically analysed, and the results (Table 1) show that it is not normal, because of

TABLE 1. STATISTICAL ANALYSIS OF MORTALITY DISTRIBUTION IN "X" STRAIN MICE IRRADIATED WITH 600 r γ-RAYS (160 ANIMALS)

Parameter of distribution	Moments	Cumulants	Dispersion	Error
Mean	8·59	8·59	0·071	0·266
Dispersion	11·36	11·36	1·630	1·275
Asymmetry	2·15	81·69	0·037	0·192
Excess	266·9	—34676·15	0·146	0·381

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the extreme positive asymmetry and considerable dispersion. This may be most readily explained by considering that the curves represent several distributions. Indeed, in some cases without any special treatment of the results, it may be seen that the mortality distribution curves have several peaks. One of the types of such distribution is shown in Fig. 2. However, to reveal these "mortality peaks", as we term them, it is frequently necessary to treat the results in a special way.

To reveal and study the mortality peaks, it is necessary to analyse many-peaked curves. The problem is rather complex and there are no standard methods of solution. However, in some specialized publications, dealing with a variety of topics, some devices of analyzing similar curves are described. Harding [6] transforms percentages of cumulative mortality into probits and regards them as a function of the magnitude being measured. He judges the presence of peaks from the slope of the curve. Forbes [7] examines the increments of the logarithm of frequency and, performing a linear interpolation along the points lying on a straight line, finds the position of the peaks on the zero ordinates. The increments of the probits of cumulative mortality may also be studied, and the presence of peaks judged by the deviations from the horizontal [1]. All these methods suffer from a common disadvantage, since they are all based on the erroneous assumption that individual components of a curve or the whole curve must follow the normal distribution. This approach must therefore be abandoned. Differentiation of the distribution curve could be the simplest procedure, but is inapplicable to the practicable volume of experiments and in the absence of a continuous record of survival time.

Evaluation of the probability of death during given intervals of time furnishes satisfactory results [1]. Here the number of animals dying during an interval of time must be divided by the number of animals surviving up to the beginning of that interval. In other words, the probability p of an animal's dying on the day t is evaluated according to

$$p_t = \frac{a_t}{N - \sum_{i=0}^{t-1} a_i}$$

where a_t is the number of animals dying during the ith day, and N the total number of animals in the experiment. This method does not presume a definite form of the

components, and at the same time it reveals the presence of mortality peaks more readily than does the consideration of frequency distribution; in direct studies of frequencies, the height of the peaks diminishes proportionately to the number of animals remaining, whilst when the frequencies are referred to the number of surviving animals, the systematic displacement is removed.

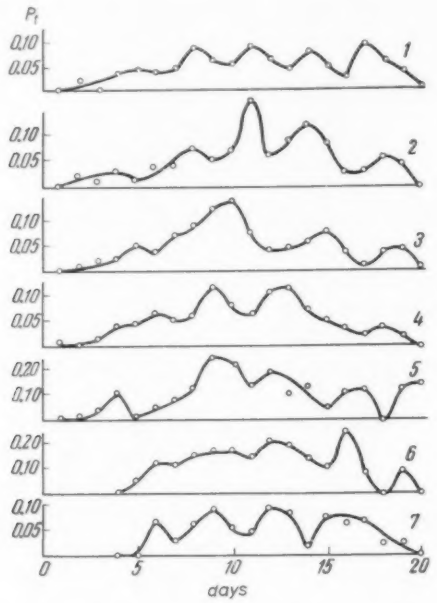

FIG. 3. Mortality peaks in mice of different strains [1–4], in rats [5], pigs [6] and monkeys [7] after X- or γ-irradiation. Abscissa: number of days after irradiation.

To establish the validity of the mortality peaks, a large number of different strains of mice of both sexes, irradiated with different doses, as well as of different species of irradiated animals, was studied. Some of the results obtained are shown in Fig. 3, which compares the mortality peaks for four strains of mice, and of white rats exposed to approximately equal biological doses (about $LD_{70/30}$). The results of treating the experiments of other authors on the irradiation of pigs [8] and monkeys [9] are also given.

Fig. 3 indicates that in all cases mortality distribution gives a series of peaks, their general character being similar, though not identical.

The problem of how the peaks change with variations in radiation doses—whether they are characteristic of all doses, and whether their height and position change—is of interest. We have therefore analysed extensive material (over 2000 animals) on the γ-irradiation of strain "H" mice. Table 2 shows the data on the number of animals in each group and on their mortality. Fig. 4 represents mortality peaks with different irradiation doses. The most interesting feature of the results

TABLE 2. INFLUENCE OF VARIOUS γ-RAY DOSES ON THE MORTALITY OF "H" STRAIN MICE

Irradiation dose (r)	Number of mice	Died by the 2nd day		Mean survival (days)
		Number	Percentage	
450	341	75	22·0 ± 2·25	12·5
600	599	356	59·4 ± 2·01	10·4
700	599	459	76·7 ± 1·74	9·7
850	418	398	95·2 ± 1·24	7·6
1000	300	300	100·0	4.4

obtained is that the time distribution of the mortality peaks is, in the first approximation, independent of the radiation dose. Even if there is any systematic displacement of the peaks, with increasing dosages, it is insignificant. Thus it may be sufficiently reliably considered that, independently of the dose, the boundary between the first and the second mortality peaks lie between the fifth and the sixth day, and the boundaries of subsequent peaks between the ninth to tenth, twelfth to thirteenth and fifteenth to sixteenth days.

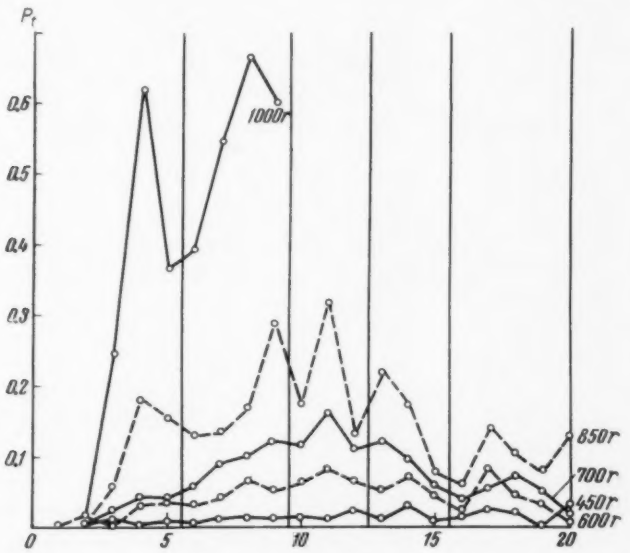

FIG. 4. Mortality peaks in "H" strain mice after irradiation with different doses of γ-rays. Abscissa: number of days after irradiation.

It is quite obvious that all peaks increase in height with increasing doses. Hence the relationship between individual peaks and the radiation dose must be determined. The number of animals dying during each of the above intervals is reasonably significant in this respect, since, although individual distributions overlap, the height and width of neighbouring peaks are fairly close, and hence their overlap is mutually

almost compensated. In the elucidation of the dependence of mortality distribution on the dosage, we employ the same procedure as in the revealing of the peaks themselves, viz. the number of mice dying during each interval of time is expressed as the percentage of the number of mice surviving until that period. Without this, all the curves, except the first one, would tend to a limit less than 100 per cent, the height of which would depend on the mortality during the preceding peaks. The dependence of mortality percentage, corresponding to each peak, on the irradiation dose is presented in Fig. 5. Two features are characteristic for the curves obtained. Firstly, the LD_{50} for all of them lies within the limits of 800–900 r. Secondly, their shape is not the usual S-form, but resembles rather a power function.

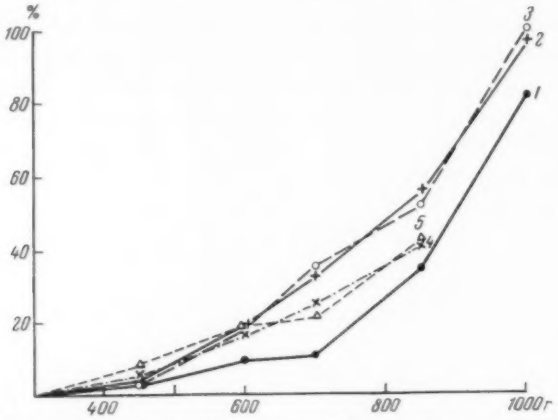

FIG. 5. The dependence on the irradiation dose of the mortality percentage in the various peaks (mice). The first, second, etc. peaks are numbered 1–5.

To use the analysis of mortality peaks as a method of investigation, it is necessary to find out how much the magnitude of the peaks depends upon experimental conditions not taken into account. For this purpose, we took 300 male strain "H" mice, irradiated at different times (from July 1953 to June 1954) with 700 r and an intensity of 50 r/min. The material was divided chronologically into two groups and the mortality distribution in the peaks was checked using the criterion χ^2 for homogeneity. The results of this analysis, given in Table 3, show there is no statistically significant difference between the two distributions. Comparison of the mortality percentage during each peak using the t-test for time also gave no statistically reliable divergencies. These results indicate that experimental conditions not taken into account do not have any significant effect on the mortality distribution. Moreover, 150 females of the same strain, irradiated under identical conditions and at the same time, were compared with both the above groups of males. Here the mortality distribution in the peaks was different from that of the males, and the divergence was statistically significant. The data in Table 4 show that the divergence is due almost exclusively to the difference in mortality during the second peak. Thus, under the usual conditions of radiobiological experiments, the distribution and height of mortality peaks are satisfactorily reproducible. At the same time, in experiments with animals known to be dissimilar (the males and females of strain "H" react

TABLE 3. MORTALITY DISTRIBUTION (PEAKS) IN TWO EXPERIMENTS WITH 700 r IRRADIATION OF "H" STRAIN MALE MICE

Period (days)	1st group		p_t	2nd group		p_t	Difference*	t	p
	Number			Number					
	Survived	Dead		Survived	Dead				
0–5	150	23	0·15 ± 0·029	150	18	0·12 ± 0·027	0·03 ± 0·04	0·83	0·41
6–9	127	51	0·40 ± 0·044	132	39	0·29 ± 0·040	0·11 ± 0·059	1·80	0·06
10–12	76	29	0·38 ± 0·054	93	40	0·43 ± 0·051	0·05 ± 0·075	0·64	0·52
13–15	47	10	0·21 ± 0·060	53	14	0·25 ± 0·060	0·04 ± 0·085	0·48	0·63
16–20	37	11	0·29 ± 0·075	39	6	0·15 ± 0·058	0·14 ± 0·095	1·51	0·12

* $\chi^2 = 6{\cdot}6$; $n = 5$; $p = 0{\cdot}25$.

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somewhat differently to irradiation) a different picture of the peaks is obtained, the difference being confirmed statistically.

All our results indicate that the time distribution of mortality in irradiated mammals gives a series of peaks. These peaks may be revealed by a reasonably simple treatment of the data. The peaks are sufficiently constant for given experimental conditions and, probably, represent a general phenomenon, since they were obtained in all cases so far analysed by us.

The question of how to interpret this intriguing phenomenon remains open to a certain extent. As mentioned before, we began the mortality distribution analysis starting from the premise that different pathological processes, with different latent periods, must be reflected in the form of this distribution.

The analysis has confirmed our expectations, and hence we naturally regard the mortality peaks as a reflection of the different terminal causes of death in irradiated animals. Although the results of the present work do not contradict this viewpoint, it will be considered as proven only after some special experiments. One type of such experiments would involve the comparison of mortality peaks under the action of various anti-radiation agents. If these agents reduce the mortality of irradiated

TABLE 4. MORTALITY DISTRIBUTION (PEAKS) IN 700 r IRRADIATION OF "H" STRAIN FEMALE MICE

Period (days)	Number		p_t	Difference w.r.t. 1st group of males*			Difference w.r.t. 2nd group of males†		
	Survived	Dead		$[p_t(♂♂) - p\,(♀♀)]$	t	p	$[p_t(♂♂) - p_t(♀)]$	t	p
0–5	150	9	0·06 ± 0·019	0·09 ± 0·035	2·64	0·008	0·06 ± 0·033	1·82	0·06
6–9	141	24	0·17 ± 0·032	0·23 ± 0·054	4·30	0·0002	0·13 ± 0·051	2·46	0·014
10–12	117	34	0·29 ± 0·042	0·09 ± 0·068	1·33	0·18	0·14 ± 0·066	2·10	0·035
13–15	83	24	0·29 ± 0·050	0·08 ± 0·078	0·98	0·33	0·04 ± 0·078	0·45	0·65
16–20	59	13	0·22 ± 0·054	0·08 ± 0·093	0·82	0·42	0·07 ± 0·079	0·85	0·39

* $\chi^2 = 30{\cdot}6$; $n = 5$; $p = 10^{-5}$. † $\chi^2 = 33{\cdot}1$; $n = 5$; $p = 3 \times 10^{-6}$.

animals through acting on different pathological processes, then if the above viewpoint is correct, some of the agents should act more or less selectively on different peaks, and substances with similar chemical and pharmacological properties should also have a similar effect on the mortality distribution of irradiated animals. Our experiments (to be reported in another publication) indicate that this does indeed occur.

When we say that the mortality peaks reflect the different terminal causes of death, we do mean *terminal* ones, since the initial link of all radiobiological reactions is the same, viz. the absorption of radiation energy by the substance. We believe that some pathophysiological processes leading to the death of irradiated animals develop as a result of divergence of the same chain of physicochemical and cytological reactions. In favour of this interpretation of mortality peaks is, incidentally, the similarity in the dependence of mortality on the dose in all the five peaks occurring to a significant extent with doses of the order of $LD_{50/30}$. Thus, we consider that the mortality peaks observed reflect mainly relatively late stages of those processes occurring in animal organisms irradiated with lethal doses of ionizing radiation.

Our results appear to us of interest primarily because they bear witness to the possibility of using biophysical analyses in those regions of radiobiology so far lying exclusively in the province of radiopathology. The study and analysis of mortality peaks—in itself a formal procedure—may, in conjunction with morphological and pathophysiological investigations, deepen our knowledge of processes occurring in an irradiated organism. Besides, mortality peaks can, in our opinion, serve as a phenomenological basis for the classification of empirical observations and for the planning of definite, more specific, experiments.

CONCLUSIONS

(1) The time distribution of mortality in irradiated animals is a many-peaked one and, with doses of the order of $LD_{50/30}$, gives up to five peaks in the course of 20 days following irradiation.

(2) An increased irradiation dosage has no effect on the time localization of the peaks, but increases their height. All the peaks show similar dependence on the dose.

(3) Different experiments give reproducible results, but the height of the second peak was found to be different in the males and females of a given strain of mice.

(4) It is presumed that mortality peaks reflect various terminal causes of death in irradiated animals.

In conclusion, we wish to express our deep gratitude to N. V. Timofeev-Resovskii, A. A. Liapunov and N. S. Chetverikov for their interest in this work and for their valuable criticism.

Translated by I. GORE

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PRIMARY AND SECONDARY LESIONS IN THE BONE MARROWS OF X-IRRADIATED ANIMALS*

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(*Received* 30 *June* 1956)

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IT is important in analysing the pathogenesis of radiation sickness to decide which of the phenomena observed are related to the initial reaction to ionizing radiations. Although some work has been done in this field the problem is still unsolved.

At the International Radiobiological Conference in Cambridge, Betz, Booz and Firket [1] touched upon the mechanism by which ionizing radiation acts on bone marrow, and noted that some workers believe that radiation possibly acts directly on the haemopoietic cells, whilst others consider the effects to be conditioned by general changes in the organism. Sipovskii [2] studied the "reflected" action of ionizing radiation on the bone marrow in rabbits. He irradiated the right hind leg of an animal, screening with lead plates areas of the body not to be irradiated. Destruction of bone marrow cellular elements was observed both in the irradiated and the non-irradiated limbs; the destruction in the latter was explained by the author as a "reflected" effect of the radiation.

Bloom [3] has studied the lesions in irradiated and in screened limbs of rabbits. Having found severe lesions in the former and insignificant ones in the latter, the author concluded that it is impossible to consider these experiments as a confirmation of humoral transport of X-ray effects, taking into account the impossibility of perfect screening.

Rodesch, Jaudel, Chirpaz and Mandel [4] determined the nucleic acid content—with a separation into ribonucleic and deoxyribonucleic acid fractions—in the bone marrow of white rats during the interval of time between 2 and 21 days after a single total irradiation and after local irradiation of the left hind limb (the rest of the animal being screened with lead). The sharp decrease in the content of both acids, more marked after total than after local, irradiation, is explained by the authors to be due not only to the direct destructive effect of X-rays, on the radio-sensitive bone marrow, but also to secondary influences involved in total irradiation.

Meisel and Sondak [5, 6] have worked out methods of luminescence microscopic analysis for revealing early changes in the irradiated organism. These methods have been used in the study of early reactions to irradiation in the bone marrow of white rats and of mice. Half an hour (or less) after total irradiation, small necrotic foci of the damaged cells were observed in the bone marrow preparations; their number increased with higher radiation doses and a longer time after the irradiation, reaching a maximum and then decreasing due to resorption and destruction by macrophages.

The present work is a continuation of the above experiments. Its purpose was to elucidate, by luminescence microscopy and by ordinary histological methods, the character of the changes in the irradiated bone marrow cells, so as to establish the

* Biofizika **2**: No. 4, 495–500, 1957 [Reprint Order No. BIO 64].

direct cellular damage and the subsequent processes in bone marrow areas exposed to, and protected from, radiation. To reveal the direct effect of radiation on bone marrow, a site was locally irradiated, the rest of the body being screened.

METHOD

White rats weighing 250–320 g were exposed to a single local irradiation using an X-ray apparatus RUP-1; doses of 100, 250, 500, 1000 and 3000 r were administered under the following experimental conditions: 190 kV, 15 mA, distance 20–26 cm, filters 0·5 mm copper and 0·75 mm aluminium, dose-rate 60–112·5 r/min; in irradiations with 10,000 and 20,000 r, the dose-rate was 360 r/min (without filters); the right hind leg of the animal was irradiated, the rest of the body being screened. For this purpose, the rats were attached by the back to a stand covered with lead. The non-irradiated left hind leg, the thorax, abdomen and head were covered with a lead screen 5 mm thick. The limb to be irradiated was placed in a 10×10 cm irradiation field.

The animals were decapitated at intervals of from 18 sec to 0·5 hr and 1, 2, 3, 4, 6, 10, 12, 16, 18 and 24 hr after irradiation. Smears were prepared from the fresh, non-fixed marrow of the irradiated and non-irradiated limbs and these were stained on the slide with mixtures of fluorochrome solutions by the method of Meisel and Sondak [5]. The main staining solutions were prepared in 1: 1000 dilutions. The following staining mixtures were used: No. 1, acridine orange 0·5 ml, basic fuchsin 0·1 ml, physiological saline 10 ml; No. 2, acridine orange 0·5 ml, Congo red 0·5 ml, basic fuchsin 0·05 ml, physiological saline 10 ml. A mixture of acridine orange and basic fuchsin was used for studying changes induced by radiation in the cells before and after their death.

Treatment with acridine orange results in the nuclei of various cells luminescing with different shades of green and yellow. Granules in the protoplasm of leucocyte-type cells luminesce bright-red. Addition of basic fuchsin to acridine orange enhances the brilliance of the luminescence of affected necrotic foci.

The mixture of acridine orange, fuchsin and Congo red was used for the partial extinction of undamaged cells, so as to reveal more clearly micronecroses and isolated affected cells. Bone marrow smears, treated with the above fluorochromes, were subjected to luminescence microscopy under blue light. The number of micronecroses per mg of bone marrow was determined, as well as the number of micronecroses, and the total number of affected cells per 1000 cells of bone marrow elements. The experiments were carried out on 162 animals.

RESULTS

Examination of bone marrow preparations treated with fluorochromes (acridine orange plus fuchsin plus Congo red) reveals a general dull, dark-green luminescence. Against this background are seen the bright light-green luminescing affected cells and foci of such cells—micronecroses, the description and microphotographs of which have appeared in the paper of Meisel and Sondak [6].

Mitchell [7], in the paper read at the International Conference on the Peaceful Uses of Atomic Energy, reported work wherein, using ultra-violet microphotographic methods, an increased nucleic acid absorption, especially in the cytoplasm, was found 15 min after irradiation of lymphocytes and lymphatic tissue of adult rats with a total

of 75–1200 r; these biochemical changes occurred before the onset of any morphological ones.

We found morphological changes and impairment of nucleic acid metabolism in the nuclei and cytoplasm of cells in irradiated areas of bone marrow in rats decapitated only 30 sec after irradiation. Micronecroses and isolated affected cells were revealed by the staining of bone marrow preparations, from irradiated (500 r) areas with a mixture of acridine orange, fuchsin and Congo red, 18–30 sec after irradiation. To reveal the number of affected cells, preparations from the same animals were stained with acridine orange and fuchsin (without Congo red); this showed that of 1000 bone marrow cells, the protoplasm of 200–250 cells luminesced red. Nucleoproteins in the nuclei of unaffected granulocytic cells luminesced pale-green, forming small or medium-sized granulations. Their membranes luminesced whitish-green and the protoplasm dark green. Protoplasmic nucleic compounds form granular complexes which luminesce flame-red; the protoplasm of macrophages luminesces brown, of haematoblasts pink, of megakaryocytes and of reticulo-endothelial cells dark green.

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In the erythrocyte series, mature erythrocytes do not luminesce, in agreement with the findings of Bobrov [8], Meisel and Sondak [9] and Kosenow [10], whilst reticulocytes luminesce bright red.

The number of micronecroses per mg bone marrow, on local irradiation, increases with increasing time interval after irradiation. Thus, immediately after irradiation with 500 r there were 12 micronecroses per mg, after 0·5 hr, 1 hr and 2 hr there were 63, 86 and 578, respectively; thereafter their number began to decrease.

It was found that the number of micronecroses per mg bone marrow increases with increasing irradiation dose (Figs. 1, 2).

We have also studied the dependence of the number of micronecrotic foci and the total number of affected cells in them, per 1000 bone marrow cells, on the radiation dose and the time elapsed since the irradiation. A directly proportional relationship was found to exist between these factors and the number of affected cells

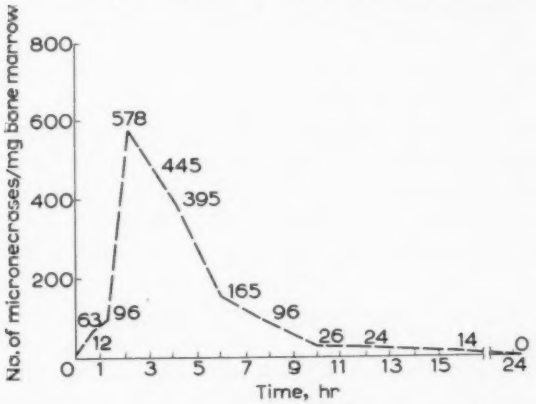

FIG. 1.

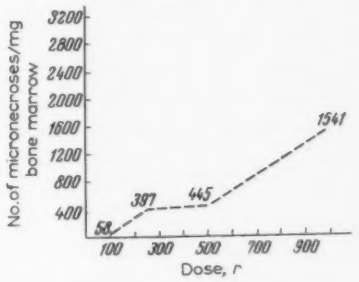

FIG. 2.

FIG. 1. Time variation in the number of micronecroses per mg bone marrow from a locally irradiated (500 r) limb.

FIG. 2. Number of micronecroses per 1 mg bone marrow 3 hr after local irradiation of a limb (dependence on dose).

TABLE 1. NUMBER OF MICRONECROSES, PER 1000 CELLS OF BONE MARROW ELEMENTS, AND THE TOTAL NUMBER OF AFFECTED CELLS IN THEM, DEPENDING ON RADIATION DOSAGE AND THE TIME ELAPSED SINCE THE IRRADIATION*

Dose (r)	Time elapsed since irradiation (hr)							
	1	2	3	4	6	10	12	24
100†	—	14/33	31/77	10/22	5/13	2/4	2/4	0
250	—	22/73	38/156	15/47	32/72	7/14	4/8	0
500	5/10	32/78	43/190	19/58	18/53	15/54	14/50	0
1000	7/14	33/80	49/342	23/121	19/71	15/43	12/37	0
3000	12/24	39/85	57/414	48/283	27/82	15/43	12/40	0
10,000	12/24	55/130	56/479	56/448	56/194	24/89	13/62	0
20,000	19/66	113/665	133/894	128/794	91/493	31/184	18/88	0

* Numerator: number of micronecroses; denominator: total number of affected cells in them.
† The animals were irradiated with 100–3000 r at a dose rate of 112·5 r/min.

in the micronecroses. The number was a maximum 3 hr after irradiation, but decreased later due to resorption and destruction by macrophages (see Table 1).

Studies of bone marrow preparations, from screened limbs, stained similarly to the irradiated ones, showed no micronecroses after any dose or any interval of time. From this it may be concluded that the screening was effective and the reactions described were produced by the local action of radiation. Figs. 3 and 4 show respectively the presence and absence of micronecroses in the irradiated, and in the shielded, bone marrow.

It must be noted that during early structural changes in the cells of irradiated bone marrow, the following phenomena are observed: the nuclei of granulocyte cells increase in size; the fine and medium granulations of nucleoproteins and nucleoli liquefy; the nuclei luminesce bright greenish-yellow. As a result of an accumulation of ribonucleic acid the protoplasm of these cells luminesces red; the haematoblast protoplasm luminesces brick-red. Cytolysis, or destruction of cells by macrophages, is observed. Destroyed haematoblasts are seen in the macrophage protoplasm. The structure of the megakaryocyte nuclei is damaged, the threads connecting the lobes

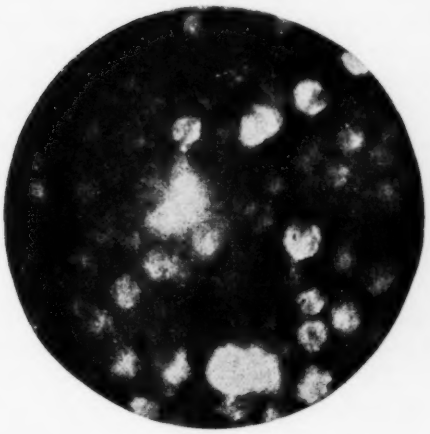

FIG. 3.

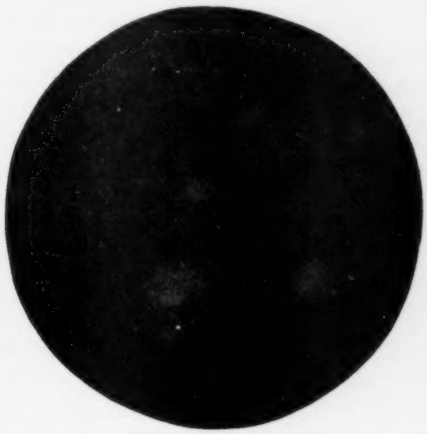

FIG. 4.

FIG. 3. Luminescent necrotic foci in the bone marrow of rat no. 120 0·5 hr after local irradiation of the limb with 500 r.

FIG. 4. Bone marrow of the screened limb of rat no. 120.

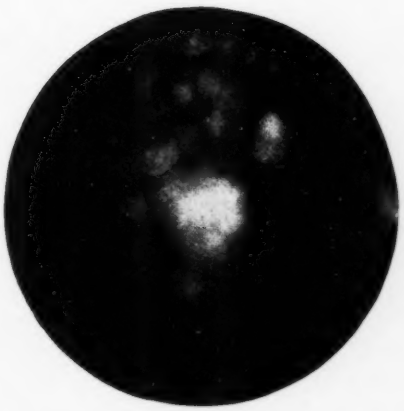

FIG. 5.

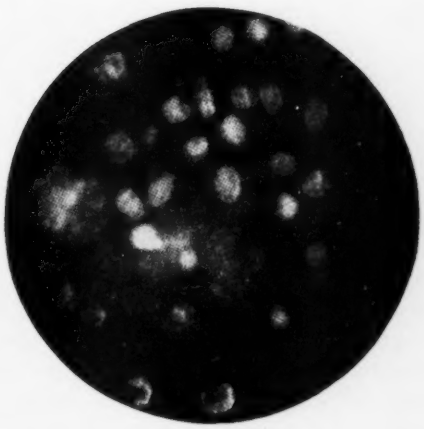

FIG. 6.

FIG. 5. Nuclear fragmentation of a megakaryocyte in the bone marrow of a rat 3 hr after local irradiation with 500 r × 200.

FIG. 6. Lysis of megakaryocyte protoplasm in the bone marrow of a rat 0·5 hr after local irradiation with 500 r.

of the nucleus dissolve and fragmentation of the nucleus and of the protoplasm occurs. The protoplasm of the megakaryocytes and of erythroid cells luminesces brick red. Destructive processes in the nucleus and in the protoplasm do not always occur simultaneously. In some instances, vacuoles appear in the protoplasm, followed by fragmentation and disintegration, with preservation of the nuclear structure; hollow nuclei remain. In other instances the process starts in the nuclei: they swell and fragmentation and karyolysis follow. Fragments of nuclei are found in the protoplasm, the structure and normal luminescence of which remains preserved.

All the above changes have been studied in detail, using the luminescence method, by Meisel, Kondratieva and Emel'ianov [11] in tissue cultures of tumour cells from X-irradiated mice.

Ordinary histological examination of irradiated bone marrow showed, in cells of erythroid type, poikilocytosis, nuclear vacuolation of erythroblasts and haemolysis of erythrocytes.

In the study of changes occurring in the affected cells at times more remote from the irradiation, the copiousness of damaged cells is noteworthy. Four to six hours after irradiation, almost all the cells are affected: they increase in size, the nuclei swell, the protoplasm undergoes lysis; there is mass phagocytosis; macrophages are seen containing large numbers of damaged cells in the protoplasm or fragments of lysing cells. The affected erythroid cells luminesce brick-red.

In addition to the above cell damage, mitosis in unaffected cells ceased in the first few hours after irradiation and was restored 6 hr after irradiation with 100–1000 r. According to Barakina [12], mitosis was restored 6 and 8 hr, respectively, after total irradiation with 700 and with 1000 r.

Luminescence and ordinary morphological studies of preparations from screened areas of bone marrow revealed the presence of mitosis 1 min, 0·5 hr and 1 hr after irradiation. Damage was observed only in isolated cells (haemocytoblasts, megakaryocytes) 2–4 hr after irradiation. Six hours after irradiation, cell damage (in the granulocyte and erythroid types) was observed identical with that occurring in irradiated bone marrow 0·5 and 1 hr after irradiation. The only difference was the absence of micronecroses. Mitosis in the screened bone marrow and the cell damage, appearing at the second to fourth hour after irradiation, point to the secondary nature of these effects, arising from the penetration of fission products from irradiated into non-irradiated areas of the body.

An overwhelming majority of workers, including Lebedinskii [13] consider that a special humoral pathway of toxic effects is possible. They are believed to originate in the products of cellular disintegration, acting on the whole organism.

Current views on the radiosensitivity of megakaryocytes are contradictory. Sipovskii [2] has noted that, in the rabbit, 24 hr after irradiation with 1800 r, megakaryocytes are destroyed. He considers these cells to be radiosensitive. Lamson and Tullis [14] regard megakaryocytes as the most X-ray resistant cells: they show little morphological change and their number decreases slightly only towards 30 hr after irradiation. Barrow and Tullis [15] noted megakaryocyte damage 48 hr after irradiation. Bloom [3] considers that erythroblasts are affected first, then granulocytes and later still megakaryocytes. Megakaryocyte damage in the first 24 hr is regarded as accidental.

We have observed megakaryocyte damage as soon as 0·5 hr after irradiation with 500 r. The luminescence of the protoplasm changed from greenish-brown to bright red. More acute changes occurred with greater doses of radiation at longer intervals after irradiation, the protoplasm luminescing brick-red. Figs. 5 and 6 show nuclear fragmentation and protoplasmic lysis in megakaryocytes 0·5 and 3 hr, respectively, after local irradiation with 500 r.

CONCLUSIONS

(1) Luminescence microscopy studies of the action of local X-irradiation of one limb have shown that micronecrotic foci, consisting of affected cells, appear in the bone marrow of the irradiated limb. No micronecrotic foci occurred in the bone marrow of the screened limb. This points to micronecroses as an early sign of direct radiation damage.

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(2) Mitosis, observed in the screened limb 1 min, 0·5 and 1 hr after irradiation, and destructive effects appearing considerably later than in the irradiated limb, indicate that this phenomenon in the screened limb must be a secondary effect arising from penetration of fission products.

(3) The nuclear fragmentation, cytoplasmic lysis and changes in the luminescence colour of nuclei and cytoplasm of megakaryocytes observed 0·5 hr after irradiation with 500 r show these cells to be sensitive to ionizing radiation.

Translated by I. GORE

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SOME DEVELOPMENTAL REGULARITIES IN THE PROGENY OF FEMALES γ-RAY IRRADIATED DURING PREGNANCY*

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(*Received* 1 *August* 1956)

IT has often been stated that the reaction of an embryo to external factors depends on its age. These differences in sensitivity are clearly related to differentiation and tissue and organ development. Detailed study shows that definite specific periods exist in various groups of animals. These periods are determined by physiological processes related to nutrition and respiration in the foetus and by developmental morphogenetic processes. Shmidt has dealt with these periods [1].

There is much in common between these processes in different groups of animals. Vernidub [2, 3] has shown that fish embryos react differently to external influences according to their age and certain sensitive ("critical") periods were observed. L. and W. Russel [4, 8] have also shown that embryos are of varying sensitivity to a given external factor at different stages of development. Using X-rays they showed that a given dose reaching the foetus (in the mouse) had different effects, according to the stage of pregnancy. They studied X-ray sensitivity in the three main periods found in mammals: pre-implantation (0–5 days in mice), main organogenesis (6–13 days) and foetal (from 14 days to birth).

They established the irradiation reaction in each period and showed that: (1) pre-implantation irradiation could cause prenatal death, but did not cause abnormalities in the surviving foetus; (2) less prenatal death was produced by second-period irradiation, but there were many still-births and abnormalities in the litters; (3) irradiation in the last period caused no prenatal deaths and almost no still-births.

This work has been confirmed by several other workers. Thus, several workers have irradiated on the ninth or tenth day of pregnancy and found the second period to be the most sensitive [9–11]. Hicks [12], who studied nervous system damage due to foetal X-irradiation in detail, also found the second period the most sensitive. In an extensive review L. Russel [13] states that the action of penetrating radiations on pregnant females, and the resulting embryological changes induced, deserve much attention. Isachenko [14] used ovicell transplantation techniques and studied the mechanism of X-ray action on the foetus to elucidate some of the relations between mother and foetus.

Penetrating radiations form a powerful means of studying embryogenesis experimentally. Another important but less studied topic is the relation between the effect on the pregnant mother and the postnatal development of the offspring. It has been stated that there are such direct relationships, but the subject has been insufficiently examined. Some workers have observed defects in the adult animals (damage to gonads, nervous system, etc.) born from mothers irradiated during pregnancy.

* Biofizika **2**: No. 4, 501–512, 1957 [Reprint Order No. BIO 65].

Hicks [12] has given detailed descriptions of nervous system defects in such animals of various ages (up to 1–5 years).

In none of the papers of which we are aware has the effect of irradiation during foetal stages on postnatal life been studied systematically. A solution to this problem could throw light on many aspects of ontogenesis.

In this work we used penetrating radiation (as being a precision method of study) by irradiating the mother at various stages of pregnancy and following the postnatal development of the offspring. As preliminary criteria we took the degree of viability, weight changes and morphological abnormalities. The latter are not analysed in detail as these changes form the subject of further study.

MATERIAL AND METHOD

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White mice and a ^{60}Co source were used, a GUT-Co-400 cobalt beam unit being employed. Doses of 200 r at 39 r/min were given. This dose is far below the lethal value for pregnant females. As a rule the mice did not show radiation sickness and never died, but the developing foetuses were clearly affected, as will appear.

Whole-body irradiation was used (groups of mice from the first–nineteenth day of pregnancy being irradiated, each mouse once only). In all, more than 300 mice were used, 45 of these not being irradiated (controls). More than 1000 young were obtained. Table 1 shows the numbers irradiated at the various times.

The larger numbers used in the first five days are due to the difficulty in getting any offspring from animals irradiated in this period—firstly because it is still difficult to establish that they are pregnant, and secondly because reabsorption is here most marked. The experimental and control animals were kept under identical conditions in standard 10 l glass tanks.

To establish the date of pregnancy the oestral cycle was followed and the animals put with the males at a fixed time of day in the oestrus stage. Vaginal smear tests on the subsequent day mostly indicated fertilization. Those considered pregnant were observed every day. The animals were weighed and vaginal smears taken to follow the oestral cycle. A gain in weight on days 8–9 and continuing dioestrus indicated pregnancy. The absence of weight gain, or a sudden drop in weight, indicated either absence of pregnancy or foetal reabsorption, which occurs in normals, but which is quite frequent on irradiation, particularly on irradiation during the early stages. In some cases reabsorption of some of the foetuses could be detected. In these cases the weight gains were small and the immediately pre-partum weights considerably below those of the controls.

All animals shown to be non-pregnant or to have reabsorbed the embryos were dissected. The morphology of uterus and ovaries was examined (if reabsorption had occurred the uterus was enlarged and hyperaemic). The ovaries were fixed and studied histologically to reveal the true yellow bodies, which indicate that pregnancy followed by reabsorption has occurred.

The pregnant animals were separated out and kept in separate tanks. The numbers of young from both irradiated and control animals were studied systematically for 42 days *post partum*. They were weighed on days 5, 13, 21, 28, 35 and 42: details are given by Shapiro *et al.* [15].

TABLE 1. STATISTICS OF IRRADIATION OF PREGNANT MICE

Duration of pregnancy prior to irradiation (days)	No. irradiated	No. giving birth	No. of young produced	No. of young surviving for 42 days
1	25	8	24	24
2	19	3	13	13
3	31	7	22	12
4	22	5	28	26
5	15	4	25	17
Total for period one	112	27	112	92
6	10	4	25	13
7	10	5	34	21
8	18	8	15	8
Total to period two (I*)	38	17	74	42
9	24	11	51	6
10	20	9	29	—
11	10	10	46	4
12	7	7	40	—
Total for period two (II*)	61	37	166	10
13	9	8	39	18
14	12	9	56	34
15	12	11	68	39
16	11	11	74	58
17	8	8	56	26
18	11	11	68	48
19	6	6	37	16
Total for period three	69	64	398	239
Total	280	145	750	383
Control	45	45	301	280

* The Roman figures denote subperiods: I—irradiation on the sixth-eighth day, II—on the ninth–twelfth day.

RESULTS

The data collected showed that the irradiation time had effects on both foetal and post-natal development. In describing the results the three periods indicated above were utilized, with corrections as seemed appropriate.

Period one irradiation caused high foetal mortality. This usually appeared as complete or partial reabsorption of the whole litter. By partial is implied litters of mean numbers less than in the controls.

It is normally difficult in complete reabsorption to establish that pregnancy occurred at all, but use of the above technique (weighing and searching the ovaries for yellow bodies and examining the uterus for thickening and hyperaemia) enabled us to establish whether pregnancy had occurred or not almost entirely without possible error.

In all, 112 animals were used in period one but only 27 gave litters. Complete reabsorption was shown in 70 per cent (see Fig. 1: the vertical lines on all figures denote the ends of periods). The mean litter numbers were less than in the controls, averaging 4·5, control being 6·7 (Fig. 2). This indicates partial reabsorption. The absence of still-births in this period is noteworthy.

The weights and survival of these litters were recorded up to the forty-second postnatal day: these data revealed some features of postnatal development. The first period shows typically very little postnatal death. Of the 112 young produced, 92

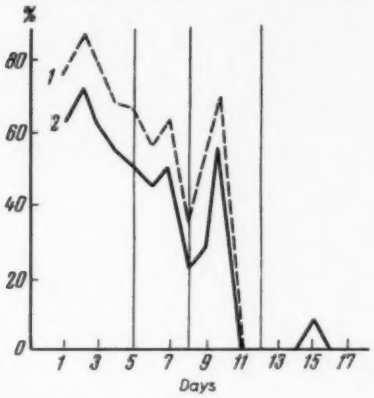
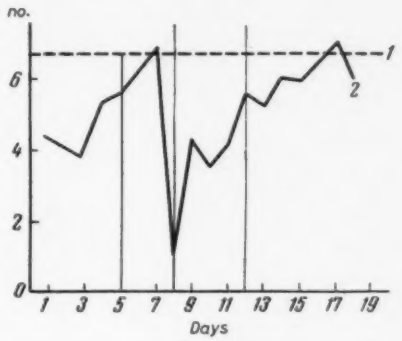


FIG. 1.



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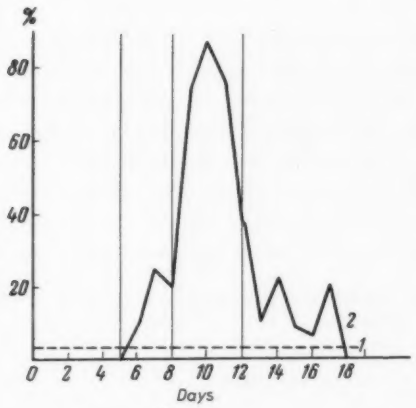

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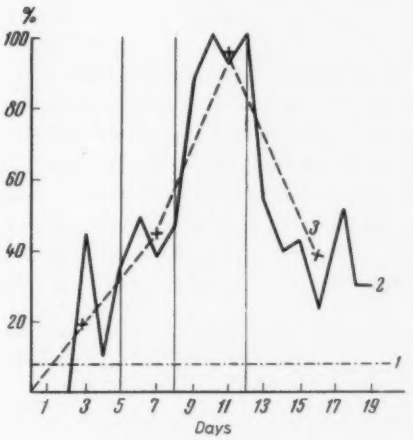

FIG. 4.

FIG. 1. Foetal deaths (percentage reabsorbed). Abscissa: day of pregnancy on irradiation; ordinate: percentage of animals. (1) percentage apparently non-pregnant; (2) percentage showing reabsorption.

FIG. 2. Mean litter size. Abscissa: day of pregnancy on irradiation; ordinate: litter size. (1) control; (2) number of animals involved.

FIG. 3. Stillbirths, percentage. Abscissa: day of pregnancy on irradiation; ordinate: stillbirths, percentage. (1) control; (2) stillbirths.

FIG. 4. Postnatal deaths (in subsequent 42 days). Abscissa: day of pregnancy on irradiation; ordinate: percentage postnatal deaths. (1) control; (2) percentage postnatal deaths; (3) mean mortality per period.

(82 per cent) survived to day 42 (Fig. 4), most of the deaths occurring in the first five days, and only very few between days 13 and 42. The survivors showed no external abnormalities.

The weight data were analysed by comparing the weights of the litters with those of control litters of equal numbers (Table 2): the weights of the mice in litters of unequal numbers were different, there being also differences as against the controls (Fig. 5). For convenience and improved statistical reliability, litters closest in weight were grouped together as having 3–5 and 6–8 individuals.

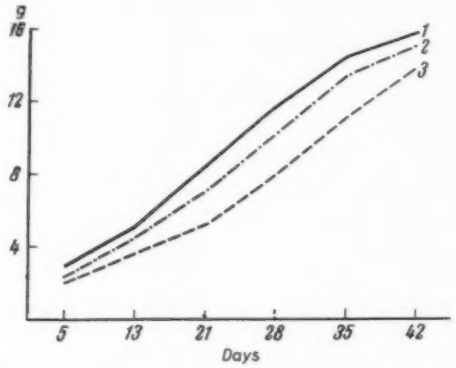

FIG. 5. Weights of controls for various litter sizes. Abscissa: age of young in days; ordinate: weight in grammes. (1) 3–5 in litter; (2) 6–8 in litter; (3) 9–11 in litter.

The control litters often exceeded eight in number; also some of the control young were not weighed throughout the 42 days as some of the females were used in the experiments and the litters transferred to other females, the experimental conditions being thereby changed. Hence, not all the control weights are given in Table 2, but only those of 140 individuals. Table 2 shows that the offspring from mothers irradiated in the first period were of weights equal to or greater than those of controls with litters of equal numbers. This difference is most clearly seen at the later stages. Zhigach's diploma work, carried out on rats in our laboratory, gave similar results.

TABLE 2. WEIGHTS OF YOUNG DERIVED FROM FEMALES IRRADIATED IN PERIOD ONE (DAYS 1–5 OF PREGNANCY) AND FROM CONTROLS (GROUPED BY LITTER NUMBERS)

Litter size	Reading	Numbers in litters	Mean weights (g) and time (days)						Mdif ± mdif
			5	13	21	28	35	42	
3–5	Irradiated	42	3·15	5·57	9·11	12·62	16·43	17·86 ± 0·39	
	Control	13	2·89	5·48	8·16	11·88	14·56	15·39 ± 0·83	2·47 ± 0·91
	Irradiated: % of control		109	102	112	106	113	116	
6–8	Irradiated	49	2·24	4·75	7·26	10·89	13·8	15·91 ± 0·32	
	Control	127	2·31	4·79	6·99	10·21	12·61	15·27 ± 0·24	0·64 ± 0·79
	Irradiated: % of control		97	99	104	107	109	104	

Although it is as yet difficult to explain the effect it is of interest and demands further study.

Period two irradiation. L. and W. Russel [10, 11] state that this period lasts from the sixth to thirteenth days. The data show that the period should be subdivided as far as radiation reaction is concerned, as irradiation on days 6–8 produces results so different from those for days 9–12. The period should be subdivided into two sub-periods. This appears particularly clearly when the foetal stage is studied. Irradiation on days 6–8 causes less reabsorption than on earlier days (of 38 animals, 17 gave birth). The fractions reabsorbed for days 6 and 7 are 44 per cent and 50 per cent respectively (Fig. 1) and the overall numbers in the litters rise. On day 8 the total reabsorptions are still further reduced but partial reabsorption occurs and the mean litter size is reduced. Day 6 is the first to give still-births (Fig. 3).

The offspring from animals irradiated on days 6–8 developed normally; of 74 born, 42 (56 per cent) survived to day 42, all three days giving comparable results. The young are of weight somewhat below the controls (Table 3).

The effects produced by irradiation on days 9–12 are quite different. A total of 61 animals was used, 24 on day 9, 20 on day 10 and 17 on days 11 and 12. Prenatal deaths again begin to rise for day 9, being particularly high for day 10, reaching 53 per cent, but the deaths occurred much later than previously.

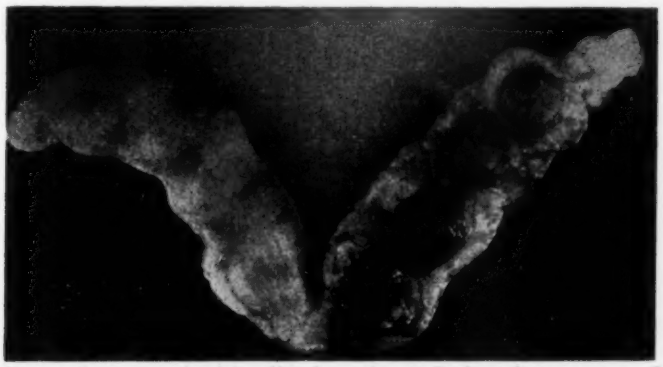

FIG. 6. Uterus from an animal irradiated on the tenth day of pregnancy. On right reabsorbed embryos can be seen (late reabsorption).

TABLE 3. WEIGHTS OF YOUNG DERIVED FROM FEMALES IRRADIATED IN SUBPERIOD I (DAYS 6–8) AND FROM CONTROLS (GROUPED BY LITTER NUMBERS)

Litter size	Reading	Numbers in litters	Mean weights (g) and time (days)					
			5	13	21	28	35	42
3–5	Irradiated	8	2·41	6·5	9·1	13·05	15·35	16·94
	Control	13	2·89	5·48	8·16	11·88	14·56	15·39
	Irradiated: % of control		86	118	110	109	103	110
6–8	Irradiated	42	2·02	4·15	5·83	8·88	11·96	13·86
	Control	127	2·31	4·79	6·99	10·21	12·61	15·27
	Irradiated: % of control		97	86	84	87	96	92

When animals showing signs of reabsorption were dissected 6–7 embryos were found on days 9–10 in the uterine wall, these having the form of dense spheroidal bodies (Figs. 6 and 7) or sometimes completely formed, but dead, embryos (the mothers never died in such cases). Animals irradiated on day 10 also gave small litters (3–5). This is because few survived after birth, 2–3 being born and then dying rapidly. The mothers were then dissected and unborn embryos, up to 5–6 in number, were found in the uterus. Thus the fall in litter size is related to late death.

No reabsorption was found in the case of irradiation on the eleventh or twelfth day, and 100 per cent litters were produced.

FIG. 7. Reabsorbed embryos of spheroidal form dissected out.

In addition to foetal death due to irradiation on days 9 and 10 there were many still-births (up to 75 per cent for day 9 and 86 per cent for day 10). Day 11 shows less, but still numerous, still-births.

The young born alive showed very high postnatal mortalities from irradiation on days 9 and 10; 37 litters, containing 166 mice, showed only 10 surviving to day 42 (16·6 per cent), six being from two litters resulting from day 9 irradiations and four from one litter from day 11 irradiation. All of the young born alive after irradiation on days 10 or 12 died. For irradiation on days 9 and 10 most of the postnatal deaths occurred on the fifth to thirteenth postnatal days, the corresponding figures for 11 and 12 day irradiations being the thirty-fifth and thirty-sixth postnatal days.

Thus, irradiation in this period is lethal to both foetus and offspring born alive, most deaths of the latter being in the early postnatal period. The sensitivity for day 10 is particularly high, all the litters dying in the first postnatal day. Since few were alive throughout this day it was difficult to derive weight data.

The data show that this second period, described in the literature as that in which the main organ differentiation occurs, is not of a single nature and that days 6–8 must be considered as a separate period or subperiod. On days 9–12 vitally necessary organs are forming and damage to these has a fatal pre- or postnatal outcome. The process is evidently only beginning on days 6–8.

Irradiation in period three (days 13–19) gives a very different picture of offspring viability. Early reabsorption is absent, and only one case of late reabsorption was seen. Instances of what may be termed abortions were seen in which still-births with unseparated placentas were produced 2–3 days before the normal date. These were also found in mice irradiated on days 15–17 in single instances, the rest surviving to birth, the litter size being as in the controls (Fig. 2).

The postnatal viability was here much higher than in the previous case, 60 per cent surviving to day 42 (Fig. 4).

The young from the irradiated animals weighed less than the controls (M_{dif}: $m_{dif} = 9{\cdot}0$), the difference being significant when litters of 6–8 are considered (Table 4). There was no difference in litters of 3–5, but this cannot be considered statistically significant (M_{dif}: $m_{dif} = 0{\cdot}35$) since the total number of young was very small (20).

TABLE 4. WEIGHTS OF YOUNG DERIVED FROM FEMALES IRRADIATED IN PERIOD THREE (DAYS 13–19) AND FROM CONTROLS (GROUPED BY LITTER NUMBERS)

Litter size	Reading	Numbers in litters	Mean weights (g) and time (days)						Mdif ± mdif at day 42
			5	13	21	28	35	42	
3–5	Irradiated	20	2·58	5·06	8·31	11·26	14·0	15·97 ± 0·71	0·58 ± 1·068
	Control	13	2·89	5·48	8·16	11·88	14·56	15·39 ± 0·83	
	Irradiated: % of control		89	92·5	100	94	95	104	
6–8	Irradiated	219	1·7	3·9	5·64	9·0	9·97	11·99 ± 0·21	3·28 ± 0·31
	Control	127	2·31	4·79	6·99	10·21	12·61	15·27 ± 0·24	
	Irradiated: % of control		82·5	82	81	89	79	78	

Irradiation in this period thus has less effect on the viability. Postnatal deaths are much less frequent and mostly occur later. But the young are weaker, as is shown by the lower weights and general failure to develop (in some cases the offspring were obviously dwarfed). This is due to period three being one of rapid growth retarded by irradiation. The retardation appears also in the postnatal growth. Certain types of radiation damage peculiar to mice are also produced in this period, namely, bone distortion, late eye opening and in some cases blindness, which are generally fairly rare in mice.

DISCUSSION

The data presented agree with those in the literature. Irradiation with ^{60}Co γ-rays at various periods of pregnancy in mammals (mice, rats, etc.) has specific effects on foetal development. The data also show that the effects exerted at various stages in prenatal life are also specifically reflected in postnatal life.

A more detailed picture of the way γ-rays influence the foetal development at various periods can be gained from postnatal studies. It is therefore inadequate to study prenatal effects alone, as some have done.

The relation between pre- and postnatal development indicates how the influences act at the various prenatal times and how this is reflected in postnatal growth and development. This is extremely important in clinical studies of irradiation during pregnancy and the effects on development this may have. Its theoretical importance is no less, since by disturbing the course of development, even with agents as powerful as γ-rays, we may deepen our understanding of ontogenesis. It can thus be shown that the effects on development due to any agent depend on the time of application.

It has been shown that fairly sharp boundaries exist between the periods as regards pre- and postnatal development and growth. In the first period the embryo is very radiosensitive and reacts quickly, early reabsorption occurring. The embryos not reabsorbed are born alive and almost all develop normally up to day 42; they may

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even exceed the controls in weight, which has so far not been explained. It may be that in this period the fertile ovum is still undifferentiated and is either damaged as a whole or completely undamaged.

Period two can be subdivided on the basis of the postnatal development. The period of differentiation is one in which the organism is most radiosensitive. Deaths in this period are mostly still-births, and while it is stated in the literature that days 9 or 10 are the most radiosensitive, postnatal studies show that days 11 or 12 are no less so, the sensitivity merely appearing later, postnatal death occurring in almost 100 per cent of cases. Growth and development are most rapid in this period and hence any damage may prove lethal. The last period is the least sensitive, as regards the mortality resulting. Scarcely any prenatal deaths occur, and much fewer postnatal ones, most of the young surviving for 42 days. But the survivors are much weaker than the controls, and than the offspring from animals irradiated in period one. They show lower weights and specific irradiation damage. The depressed growth is evidently due to the last period being one of rapid growth.

Thus γ-irradiation at different periods has different effects on postnatal development, each period having a specific effect. All the data presented here indicate that γ-irradiation is an efficient method of studying effects due to prenatal agents produced in postnatal life. The data show X-rays and ^{60}Co γ-rays to produce very similar effects.

CONCLUSIONS

(1) The published data on the effects of X-rays on pregnant mice and the data on ^{60}Co γ-ray effects given here show that ionizing radiations have effects dependent on the stage of pregnancy. The radiosensitivity and radioreactions are specific to the various periods of embryo development.

(2) The postnatal development has to be studied as well as the prenatal to give a better conception of the radiosensitivity, since the postnatal effects on viability, weight and morphology vary with the irradiation time.

(3) So far three periods have been differentiated, of differing radiosensitivities: pre-implantation (0–5 days), main differentiation (6–13 days) and growth (thirteenth day to end).

(4) Single-dose whole-body irradiation at all times (from day 1 to day 19) and postnatal development studies show that the second period must be divided into two subperiods of differing sensitivity, i.e. days 6–8 and 9–13.

Translated by J. E. S. BRADLEY

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AN ARRANGEMENT FOR CYTOSPECTROPHOTOMETRY*

N. V. KOROLEV and L. S. AGROSKIN

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(*Received* 1 *March* 1957)

INVESTIGATORS usually set themselves two aims when studying the microstructures of biological preparations: (1) to resolve the finest possible details of the preparation; and (2) to determine the chemical natures of the structures seen and to measure the contents of the more important materials in an area (qualitative and quantitative cytochemistry). The second is usually dealt with by cytospectrophotometry using either the inherent absorption of the materials or the absorption due to dyes bound to the various materials to different extents. In both cases the Beer–Lambert law is used: $D = \mu cd$, where D is the optical density of the area, μ the molar extinction coefficient for the wavelength used and c the molarity, d being the absorbing layer thickness.

Cytospectrophotometers are used for spectrophotometry of cellular structures, these being a combination of microscope and spectrophotometer [1–6]. Concentrations of 0·5–5 per cent absorbing material per μ^3 (e.g. nucleic acids) can thereby be observed. The minimum detectable amount of material is as little as 10^{-13}–10^{-14} g.

The present article gives a description of an arrangement for absorptive spectrophotometry of micro-compounds. The minimum diameter of the section measured by photometer is roughly 1μ. The spectral interval is from 220 to 600μ. The exactness of measurement of the absorption is 1–2 per cent.

OPTICAL SYSTEM

In some instruments already described the preparation is illuminated in the usual way. A screen with a small hole delimits the area to be studied in the plane of the magnified image, a photocell being behind the hole. In this system scattered light is inevitably troublesome as a large area of specimen is illuminated. In our arrangement only the very small area under study is illuminated. A small aperture is inserted in the illumination train, this being focussed on the specimen. Pointwise illumination (light-probe method) eliminates errors due to non-uniform absorber distribution in the cell and reduces the scattered light.

Fig. 1 shows the optical system. The light source 1 is focused on the diffraction (echellette) grating 4 (1200 lines/mm) by the lens 2 via the slit 3, the light passing through this slit being dispersed by the grating, and the resultant spectrum being focussed on the diaphragm 6 (in which is the small hole mentioned above) by the

* Biofizika **2:** No. 4, 513–517, 1957 [Reprint Order No. BIO 66].

quartz lens 5. When the grating is rotated the wavelength of the monochromatic radiation passed by the diaphragm varies. The quartz lens 7, reflecting fluorite prism 8, and reflecting microscope objective 9, focus the hole in 6 in the plane of the specimen 10. The inclined reflecting surface in the prism is aluminized to 80–90 per cent reflectance. The light is concentrated on a very small area of the specimen by the objective and then passes to the quartz condenser 11. In the conjugate focal

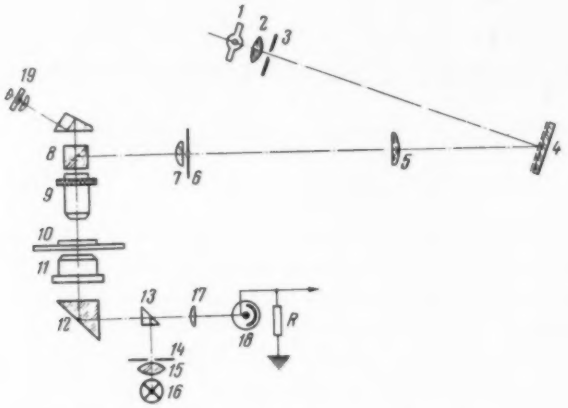

FIG. 1. Optical scheme of cytospectrophotometer.

plane, behind the right-angled prism 12, lies the lens 17 which passes the light to the photomultiplier 18. Prism 13 is removed from the light-path during photometry. Lens 17 focuses the condenser aperture diaphragm on the photocathode.

The preparation is observed and the structures to be studied set in the light probe using visible light, the preparation being illuminated by the filament lamp 16 via the train 15, 14 (diaphragm), 12 and viewed via the micrometer ocular 19. The micrometer cross-wires are conjugate with the light-probe point in the object plane. Thus, when the structure has been brought into coincidence with the cross-wires it lies in the light probe. Using a hole in 6 of 0·03 mm and a 0·8 N.A. × 65 objective, the illuminated area in the specimen (allowing for diffraction) is about 1μ in diameter.

ELECTRICAL CIRCUITS

Two types of work can be carried out with the apparatus: (1) spectrophotometry of a selected area of the specimen; and (2) optical density recording along a chosen line in the preparation, this corresponding to the distribution of absorbing material along the line.

The photoelectric circuit is used to measure the light flux passed by a point in the specimen, a photomultiplier with a U-viol window (FEU-18) being used as receiver, this being fed from a stabilized supply. The output current is amplified by an electrometer amplifier and measured with a reflecting galvanometer.

The absorption along a chosen line is measured using an electromagnetic system to scan the specimen and a facility for reproducing the curve on a cathode-ray oscillograph.

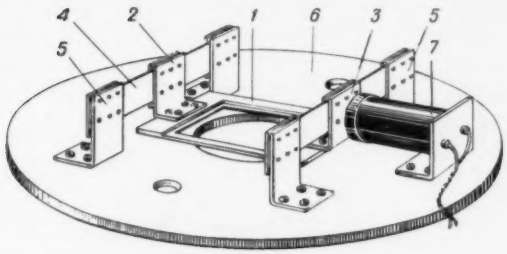

FIG. 2. Electromagnetic mechanism for transference of compounds.

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Fig. 2 shows the scanning mechanism. The specimen-holder 1 is attached by brackets 2 and 3 to two flat phosphor-bronze springs 4 and moves horizontally. The ends of the springs are fastened in the clamps 5 attached to the base 6. The base is dowelled to the microscope stage, and the specimen is set by rotating and displacing the stage. The base 6 also carries the electromagnet 7, fed from special low-frequency sawtooth source (1 cycle per 20 sec). When the core is magnetized it attracts the armature 3 and the specimen plus holder are displaced. The springs restore the holder to its original position when the voltage drops to zero. The working movement is slow and smooth, and towards the magnet, the flyback being fast and abrupt. The scan amplitude is adjusted electrically.

This design of moving system is very rigid and gives smooth scans of 20–100μ; the specimens can be held in any intermediate position and the reproducibility is adequate.

A signal from the sawtooth generator is also passed to the horizontal sweep amplifier of the oscillograph to synchronize the X-sweep and specimen motion. The photomultiplier output is amplified by a d.c. amplifier and fed to the input stage of the vertical sweep amplifier, as when using the oscillograph for recording low-frequency signals. An EO-7 oscillograph with the tube replaced by a long-afterglow 13-LO-36 was used to record the absorption distribution. Fig. 3 gives a general

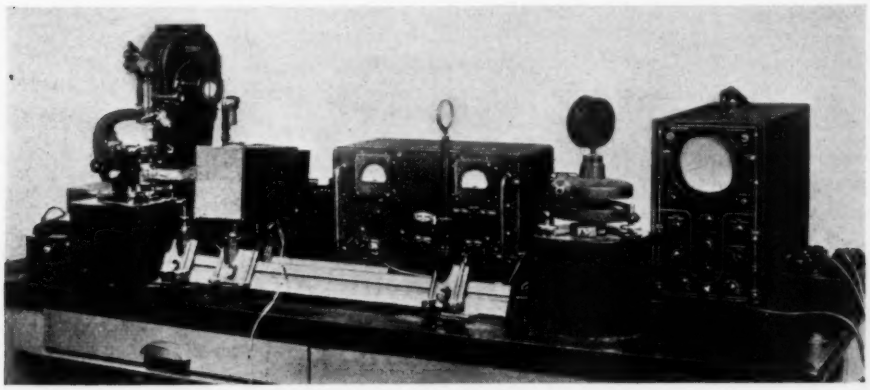

FIG. 3. General view of cytospectrophotometer.

view of the apparatus. Fig. 4 derives from a photograph of an absorption curve on the oscillograph screen taken with a human erythrocyte. The top line gives the total absorption level, the upper curve being the 405μ absorption and the lower the 313μ.

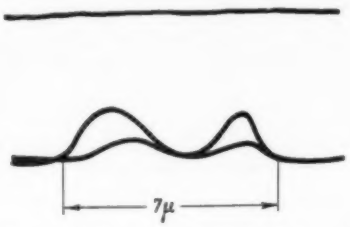

FIG. 4. Oscillogram of light absorption in erythrocytes in man (microlens 65 × 0·8).

OPERATION

The images of unstained biological objects are of low contrast in light-field conditions. This makes it difficult to detect structures and bring them into coincidence with the micrometer cross-wires. With reflecting objectives satisfactory contrast is obtainable in the visible. The condenser iris diaphragm should be closed until the specimen is illuminated by a cone of angle somewhat exceeding that of the central obstruction in the objective. Images of good contrast produced by scattering in the specimen are then obtained. This method is not very convenient, however, because the iris diaphragm must be fully reopened for photometry.

Another method of increasing image contrast (used here) is to use the fact that the field diaphragm 14 (Fig. 1) is not exactly conjugate with the specimen plane. It then has some of the properties of an aperture diaphragm and the extent to which it is open determines the aperture of the visible-light illuminating cone. By altering the size of 14 we can, as above, increase the contrast.

As light sources for the visible and ultra-violet we used the following mercury lamps: SVD*-120 (less outer glass envelope), SVD-120A, SVDSh-100, SVDSh-250, GSVDSh-120 gas discharge lamps, krypton or xenon filled, etc. The SVD-120 type was most convenient for quantitative work as its light output is very stable. When the lamp is fed via a resonant-core voltage stabilizer the intensity variations, which are the main source of error, are not more than 1 per cent. Its intensity is such that probe diaphragm diameters of 0·03 mm can be used with the following mercury lines: 578, 546, 436, 405, 365, 334, 313, 303, 297, 289, 280, 265, 454 and 248μ.

Although the SVD-120 gives a line spectrum it can be used for many purposes in cytochemical spectroscopy. The three intense lines at 280, 265 and 254μ are of most value in cytochemical work as these fall near the absorption maxima of the nucleic acids and certain amino acids.

The absorption at a given point in the specimen is measured at the various wavelengths as follows. The various spectral lines are set in turn on the hole in the probe diaphragm by rotating the grating. For each line an area of the specimen free from absorption is set on the cross-wires, and the primary reading, I_0, taken on the galvanometer. Then the structure under study is set on the cross-wires and the

* This means superhigh pressure. Translator's note.

second reading, I, taken. The optical density for that particular wavelength is $\log I_0/I$.

The probe diaphragm aperture is selected to correspond with the size of absorbing particle and objective magnification.

Fig. 5 gives optical density curves for nucleus (N) and cytoplasm (C) for mouse-

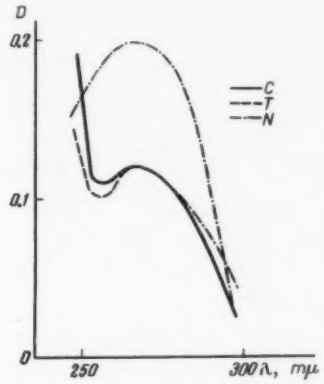

FIG. 5. Spectral curves of optic density: N—nucleus; C—cytoplasm of mouse-brain nerve cells; T—surrounding tissue.

brain nerve cells. The curve (T) relates to tissues surrounding these cells. An objective of N.A. $0{\cdot}8 \times 65$ was used. The area photometered was about 1μ in diameter. The graphs are the means of many measurements.

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INTENSITY COMPARISON FOR CERTAIN LIGHT SOURCES FOR ULTRA-VIOLET MICROSCOPY*

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(*Received* 1 *March* 1957)

SMALL ultra-violet light sources (gas-discharge lamps) are widely used for scientific and technical purposes, and for ultra-violet microscopy in particular. But the choice of a lamp is often difficult because important data such as the spectral energy distribution and the intensities in the various spectral regions are not available. We present

* Biofizika **2:** No. 4, 518–519, 1957 [Reprint Order No. BIO 67].

TABLE 1. SPECTRAL CHARACTERISTICS OF VARIOUS ULTRA-VIOLET LAMPS

Lamp type	No. measured	Wavelength (mμ)													
		436	405	365	334	313	303	297	289	280	275	270	265	254	248
PRK-4	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		62	**36**	**100**	**7**	**68**	**31**	**14**	**5**	**10**	**3**	**4**	**23**	**26**	**10**
SVD-120 A at 1·2 A	5	2·7	2·9	2·5	2·8	2·1	2·2	2·4	2·2	2·0	3·0	2·7	2·2	2·0	2·3
		67	**41**	**100**	**7**	**57**	**27**	**14**	**4**	**8**	**4**	**4**	**21**	**21**	**10**
SVD-120 A at 2·0 A	5	5·0	5·0	4·6	5·4	3·9	4·1	4·5	4·4	4·4	6·0	5·3	3·9	4·2	4·7
		67	**39**	**100**	**8**	**57**	**28**	**14**	**5**	**10**	**4**	**5**	**20**	**24**	**11**
SVD-120 A at 2·5 A	5	5·0	5·1	5·2	6·5	4·7	4·8	5·3	5·6	5·4	7·5	6·3	4·7	4·7	5·5
		60	**35**	**100**	**8**	**61**	**29**	**14**	**5**	**11**	**4**	**5**	**21**	**24**	**11**
SVD-120 at 2 A	5	4·0	4·0	3·5	4·0	3·0	3·2	3·5	3·2	3·2	4·5	4·0	3·0	3·1	3·7
		71	**41**	**100**	**7**	**38**	**29**	**14**	**5**	**9**	**4**	**5**	**20**	**23**	**11**
SVD-120 at 2·5 A	5	4·9	5·0	4·4	5·1	3·5	3·9	4·3	4·0	4·3	6·0	5·2	3·7	4·2	4·7
		69	**41**	**100**	**8**	**54**	**28**	**14**	**5**	**10**	**4**	**5**	**20**	**25**	**11**
SVDSh-104	2	90	120	45	120	30	40	55	130	40	75	30	5	0	70
		100	**75**	**80**	**14**	**36**	**22**	**14**	**12**	**7**	**4**	**2**	**2**	**0**	**13**
SVDSh-250-3	5	60	80	30	90	16	15	17	8	0·5	0	0	0	0	16
		100	**87**	**81**	**16**	**30**	**13**	**6**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
GSVDSh-120	5	3	5	1	12	1	2	4	8	4	12	7	1	1	2
		100	**86**	**48**	**37**	**32**	**30**	**27**	**19**	**19**	**18**	**14**	**11**	**12**	**10**
Philips capillary lamp	1	35	60	20	65	12	16	30	42	20	55	26	4	0	10
		100	**98**	**90**	**20**	**36**	**23**	**20**	**10**	**10**	**8**	**5**	**4**	**0**	**5**

here the characteristics of certain lamps used in ultra-violet microscopy and microspectrometry and give estimates of their spectral intensities in arbitrary units.

Lamps of Soviet manufacture, PRK-4, SVD-120 (less outer glass envelope), SVD-120A, SVDSh-104, SVDSh-250-3 and the xenon GSVDSh-120 were examined. A Philips water-cooled superhigh pressure capillary mercury lamp was also studied. A diffraction grating photoelectric microspectrophotometer was used. Fig. 1 shows the layout of the apparatus. The separate parts are labelled thus: 1 is the lamp,

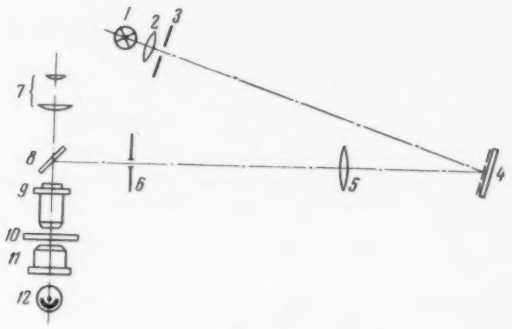

FIG. 1.

2 the condensing lens, 3 the slit (giving a 6μ spectral band), 4 the grating, 5 a lens, 6 the field stop, 7 an eyepiece, 8 a semitransparent reflecting plate, 9 a quartz-fluorite objective, 10 a stepped optical wedge, 11 a quartz-fluorite condenser and 12 a photomultiplier. This arrangement was occasioned by the interest of the author in ultra-violet microscopy.

The lamp circuits were those suggested in the manufacturers' literature, the running conditions being the approved ones. Any deviations from these (in current passed) are indicated in the table. The apparatus was adjusted so that the emitting volume was focused in the objective pupil, filling it completely. The stop 6 was focused on the wedge 10 by the objective 9. The results are expressed as galvanometer scale divisions and then referred to those for PRK-4 lamps. This type was taken as standard because of its low intensity, high stability and published spectral energy distribution.

In the table the averaged relative intensity values for 14 Hg lines are given in normal type. The values for the PRK-4 are taken as unity. Using the known spectral energy distribution for the PRK-4 those of the other lamps can be calculated. These values are given in heavy type. The highest intensity in a given wavelength range is taken as 100. The spectral energy distribution of the PRK-4 was taken from the "Illumination Handbook" (Spravochnoi knigi po svetotekhnike, Vol. 1, Akad. Nauk SSSR, 1956).

The data given are approximate since lamps of any given type differ much from one to another. The instabilities of some lamps, particularly d.c. ones, also distort the results. The table shows that under identical conditions the SVD-120A is everywhere more intense than the SVD-120. Increasing the currents drawn by the

SVD-120 and SVD-120A to 2A, which is practicable in microscopy laboratories, about doubles the intensities. Higher currents are undesirable as the brightness increase is small, while the life of the lamp is much reduced.

Translated by J. E. S. BRADLEY

A UNIT FOR SHADOWING IN THE ELECTRON MICROSCOPE*

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(*Received* 25 *January* 1957)

SHADOWING has become one of the most widely used methods for producing contrast in electron microscopy. It reveals the finest surface structural details in objects, it enables one to determine heights and—to a certain extent—the way the cross-section varies in a vertical direction. The technique requires a special apparatus comprising two vacuum pumps, a gauge, an evaporator and appropriate supplies. Shadowing demands vacua equal to those in the microscope itself (about 10^{-5} mm Hg). It would therefore seem logical to use the microscope vacuum train to carry out the shadowing. The extra pumps, etc. will then not be required and the cost of shadowing will be much reduced.

In 1952 Draganov, Eisman and Shishlovskii (Institute of Virology, Academy of Medical Sciences of the U.S.S.R.) devised a shadowing adaptor for electron microscopes. It consisted of a cylinder of about 50 cm^3 in volume containing the evaporator, specimen stage and diaphragms to prevent the evaporated metal entering the microscope. It was fitted to an EMI-2A microscope at the diffraction head.

The device, with photographs of objects shadowed, was demonstrated at the All-Union Convention on Microscopy in Leningrad in the summer of 1953, and somewhat later at a seminar on methods held by the Scientific Society at Moscow Power Institute.

In 1956 Smirnov [1] described a device to go with the EM-3. The design occasions no objection. But the photographs given to indicate the quality of the shadowing were taken at very low magnification, so the shadow deposit grain size and the sharpness of the shadows cannot be evaluated.

As to whether the device interferes with the microscope, the sole hazard is that the vapour may deposit on the working parts of the column (e.g. the pole-tips). The vapour is prevented from entering the column by using the principle, which is also involved in shadowing, that the particles of some metals, when evaporated *in vacuo*, travel in rectilinear paths. Only in this way can sharp shadows be produced. Metals giving diffusely spreading vapours cannot be used for shadowing because the shadows

* Biofizika **2**: No. 4, 520–522, 1957 [Reprint Order No. BIO 68].

produced are not sharp. So the vapour is prevented from entering the column by using simple baffles and diaphragms which intersect the path from evaporator to column in two or three places. This completely eliminates the hazard of contamination of the column. No other interference with the operation of the microscope has so far been observed.

DESCRIPTION OF THE DEVICE

Our design for the EM-3 (Fig. 1) consists of a metal cylinder with recessed ends for mounting the viewing window and fixing the mounting plate (1, 4). The mounting plate carries the evaporator leads 5. Brass collars are fixed to the evaporator electrodes to reduce the length of the heated section of the tungsten wire. The mica screen 7 prevents the mounting plate being heated. The objects to be shadowed are placed at 2.

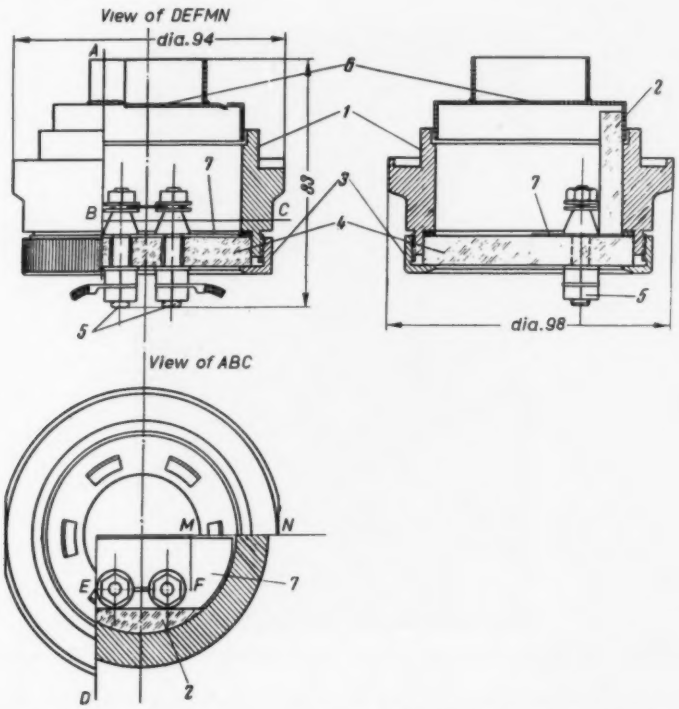

FIG. 1. Layout and sections of the unit. 1—body (duralumin); 2—object grid site (slate); 3—clamping nut (duralumin); 4—mounting plate (Perspex); 5—brass filament screw terminals; 6—screen (tinplate); 7—screen (mica).

The body bears a shaped flange which is attached to a flange on the final image chamber of the microscope via a rubber seal. This point was chosen because the shadowing head is here close enough to the diffusion pump, but not so close as to be contaminated by oil vapour. Atmospheric pressure is relied on to hold the unit in place, no extra mechanical clamping being used. A special recess cut in the flange carries a screen 6 which prevents metal vapour entering the microscope chamber.

Slits with baffles are cut in the screen to improve pump-down; no direct stream of metal vapour can pass through these.

The maximum evaporator-object distance is 35 mm. Distances of 70–140 mm are usually assumed optimal for shadowing. But in our experience there is neither deterioration in shadowing quality on reducing the distance, nor difficulty in maintaining shadowing conditions constant. The objects did not become heated.

Our experiments showed that the heated length in the evaporator can be reduced to 1·5–2 mm at wire thickness of 0·3 mm. The shadowing metal is simply hung as a wire loop on the tungsten evaporator wire held between the electrodes. Evaporators of elaborate shape (thimbles, spirals) were only needed when evaporating powders.

The usual circuits were used to supply the evaporator (6V step-down transformer, rheostat, ammeter).

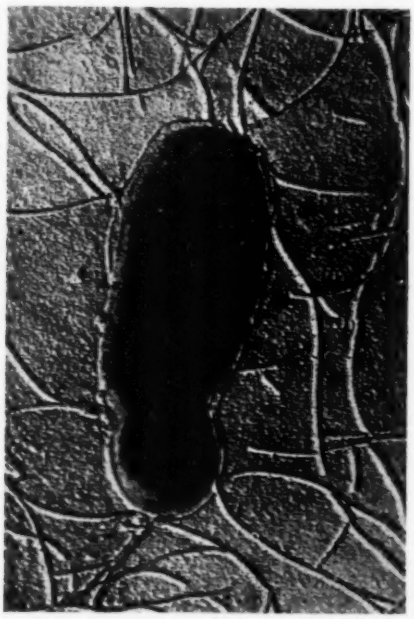

FIG. 2. Object shadowed in the device (Hertner's microbe, × 32,000, chromium shadowed).

The device is simple and convenient to use and no alterations to the microscope are required. It has been used for about four years. The operation of the microscope, so far as one can tell, has not been affected. In particular the pump-down rate is the same with or without the device. Fig. 2 shows an object shadowed in this way.

CONCLUSIONS

As the device gives satisfactory shadowing and does not hazard the microscope in any way it can be recommended for general use in electron microscope laboratories.

Translated by J. E. S. BRADLEY

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1. **SMIRNOV, A. N.,** Lab. delo 5, 1956

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THE DYNAMICS OF THE CHANGES IN THE ERYTHROCYTES DURING ACUTE RADIATION SICKNESS*

RESULTS OF A PHOTOELECTRIC INVESTIGATION OF THE KINETICS OF HAEMOLYSIS

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(*Received* 3 *July* 1956)

THE susceptibility of erythrocytes to damage by radiation, not long ago regarded as insignificant, has in recent years been demonstrated beyond doubt. A series of detailed investigations has established the great sensitivity of both young and mature erythrocytes to radiation [1–6] and quite a large part is ascribed to the erythrocytes in the pathogenesis of radiation disease. The elucidation and study of the laws of reaction of the erythrocytes during the action of radiation on the organism, beginning with the earliest stages, is acquiring a diagnostic and a prognostic significance.

Attention has recently begun to shift from the establishment of the mere fact of the susceptibility of the red blood corpuscles to damage in radiation sickness to the study of the laws of their reaction to irradiation.

The solution of this problem requires a study of the dynamics of the reaction of the red corpuscles and, in particular, needs a quantitative measurement of the phenomena that arise. Among investigations in this direction we should notice the work of Gubin using a differential erythrocytometer [4] on the dynamics of the changes in composition of the erythrocytes after the action of penetrating radiation. In this work the laws of the changes of distribution of the erythrocytes according to diameter were followed for the first time; these permitted the separation of two groups of erythrocytes and the following of their inter-relationships during the course of radiation sickness. Mention should also be made of the investigation of the stability of erythrocytes during the action of ionizing radiations on the organism carried out by Mochalina [5], who worked out a technique for the microscopic measurement of photo-haemolysis under the action of ultra-violet irradiation, and discovered early changes of photolytic stability of the erythrocytes during the radiation syndrome, indicating a rapid development of physico-chemical changes in the erythrocytes.

The changes in the red corpuscles, and in particular in their physico-chemical condition (resistance, form, rate of sedimentation, etc.) are repeatedly referred to in the handbook of radiation medicine [7]. The lack of agreement between a number of results is explicable, apparently, by neglect of the phasic nature of the changes. Nevertheless, these results together with those of the previously mentioned investigations, suggest that the recording of the physico-chemical changes may be the best method of revealing the damage to the erythrocytes by radiation, masked by the morphological simplicity of these specialized cells. We have attempted to specify

* Biofizika **2**: No. 4, 523–535, 1957 [Reprint Order No. BIO 69].

the dynamics of the changes in the red corpuscles of the irradiated organism by a differential determination of the stability of the erythrocytes, using the erythrograms described previously [8].

The method consists of the photoelectric measurement of the breakdown of erythrocytes under the influence of a haemolytic agent (in particular, hydrochloric acid). As a criterion of the condition of the erythrocytes, their power of resisting haemolysis was used. The time of action of the haemolytic agent serves as a measure of the stability. As a result of analysis, the distribution of resistance among the erythrocytes—the erythrogram—is established. By controlling the conditions (constancy of temperature and concentration of haemolytic agent) reproducible results are obtained.

The distribution of stability resistance among the erythrocytes in the normal organism is mainly an expression of their differentiation during growth; the erythrocyte enters the blood stream in a condition of maximum strength, but gradually weakens as it ripens and ages, and before its death it possesses its minimum stability. On this as basis it is possible to determine from the form of the erythrogram the duration of each stage of stability of the erythrocytes and the number of them at each growth stage at the time of investigation.

Under pathological conditions these relationships are profoundly altered. The many causes of this disturbance can be ascribed to three basal phenomena directly affecting the red corpuscles: (1) a qualitative and quantitative change in the blood-forming organs, which shows itself in the erythrogram, mainly by a change in the right-hand side from which the nature of the disturbance produced can be judged; (2) a change in the activity of the blood-destroying organs, corresponding mainly to a distortion of the left-hand side of the erythrogram; (3) the action of factors changing the physico-chemical condition of the red corpuscles in the blood circulation, which leads to more or less uniform changes of the stability of all the erythrocytes, with a corresponding displacement of the erythrogram.

Acceleration of ageing of the erythrocytes as a result of pathological erythropoiesis, or the action of toxic factors (for example, ionizing radiation) shifts the erythrogram to the left; this supports the suggestion that the erythrogram reflects not the temporal but the physiological age of the erythrocytes.

Thus the erythrogram presents a quantitative index of the condition and composition of the red corpuscles.

Photoelectric recording is an essential methodological feature of erythrograms; this makes it possible to exclude subjective and troublesome microscopic measurements of the erythrocytes. With photoelectric measurement statistical errors, connected with a small number of objects investigated, are reduced to zero, as in this case the number of cells analysed runs into tens of millions—as against some hundred by microscopic methods.

Our investigations were carried out on rabbits. The animals were submitted to whole body irradiation by the standard technique recommended by the Ministry of Health of the U.S.S.R. [9].

For the irradiation a Stabilivolt apparatus was used, run at 160 kV, current 10 mA, with 1 mm Al and 0·5 mm Cu filters. The focal distance was 50 cm and the dose rate was 30 r/min. The total doses given were 300, 400, 600, 800, 900 r. The

irradiation was carried out from the two lateral fields—half the dose from each side. The rabbit was restrained in the standard wooden cage.

Before irradiation the basal blood indices were determined for all the animals. The number of erythrocytes was determined photoelectrically as were the haemoglobin content and the erythrogram (by the standard technique at 20°). The animals were submitted to these same investigations during the whole course of the experiment.

Blood for the first analysis was taken at the moment of changing fields, i.e. after administration of half the dose. The second analysis was made immediately on cessation of irradiation. During the first hour after irradiation analyses were carried out two or three times; later at intervals of 1–6 hr to the end of the first day; and after that at intervals of 1–3 days to the end of the experiment. The blood for analysis was taken from the ear vein. Each series of experiments was accompanied by

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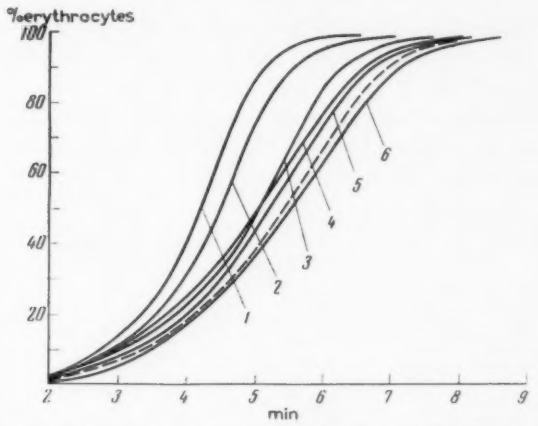

FIG. 1. Per cent haemolysis curves (loops) of rabbit L–1–3 (400 r) from the beginning of irradiation to the third day. (1)—15 min; (2)—4 hr; (3)—6 hr; (4)—13 hr; (5)—9 hr; (6)—3 days.

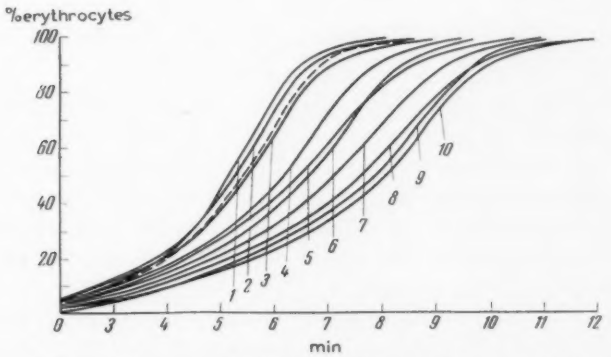

FIG. 2. Per cent haemolysis curves of rabbit L–1–3 from the 5th to the 22nd day. (1)—5th day; (2)—7th; (3)—8th; (4)—9th; (5)—10th; (6)—12th; (7)—14th; (8)—16th; (9)—21st; (10)—22nd day.

investigation of the same indices on control animals kept and investigated under conditions identical with those of the experimental animals.

The experiments showed that the condition and composition of the blood of the animals damaged by the penetrating radiation suffer regular changes that arise even during the course of irradiation, and are continued to the end of the disease. This is reflected in the kinetics of the haemolysis. In Figs. 1–3 are given the haemolysis curves in percentages for rabbit L–1–3, which had received 400 r.

The haemolysis curves during the time of the disease accomplish an almost complete period of revolution.

During the first half-period two phases occur. The initial phase is characterized by an accelerated displacement of the curve to the left. In rabbit L–1–3 (Fig. 1) it attains an extreme left position as soon as 15 min after the irradiation. In other cases this phase develops more slowly, and the extreme position is attained later (for example, in rabbit L–3–2 2 hr and in rabbit L–3–1 5 hr after irradiation). The phase of left displacement is replaced by a phase of gradual return to the initial position (Fig. 1). In our experiments this phase is considerably more prolonged than the first (from 8–14 days).

Later, the second half-period begins and is characterized by a swing of the curves to the right of the mean position (Fig. 2). This half-period also consists of two phases —a phase of displacement towards the right and a return phase (Fig. 3). The

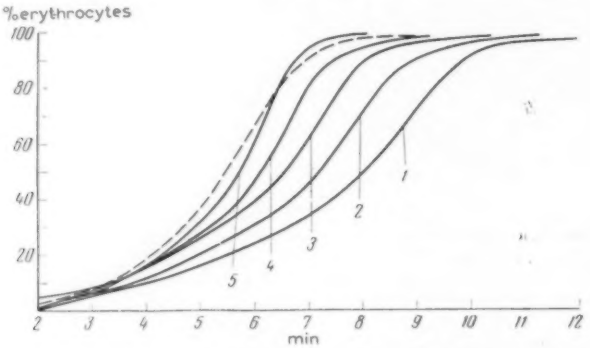

FIG. 3. Per cent haemolysis curves of rabbit L–1–3 from 22nd to 28th days. (1)—22nd; (2)—23rd; (3)—24th; (4)—26th; (5)—28th day.

durations of these phases show larger variation between individuals than do the phases of left deviation. This may be explained by a difference in the nature of the changes in the first and second half-periods. The first half-period (left displacement) apparently is linked with a direct action of the radiation, while the second half-period (right displacement) reflects the pathological and recovery processes arising after the injury. Thus the second half-period stands in close dependence on the very variable individual peculiarities of the animals.

It appears that in the first half-period the curve suffers displacement, but preserves the general features of its form. This points to more or less uniform changes of stability experienced by the whole mass of erythrocytes. With the transition to the second half-period, together with a displacement there is an actual change of shape of

the haemolysis curve. It is obviously extended, and, as it were, lies along the abscissa in the first phase, becoming normal again in the second phase of this half-period. The change of form of the curve indicates that in the second half-period the composition of the erythrocytes becomes very heterogeneous. Together with erythrocytes of low stability there appear highly stable ones resisting haemolysis up to 12–16 min instead of the normal 8–9 min. This result agrees with Frank's conclusion,

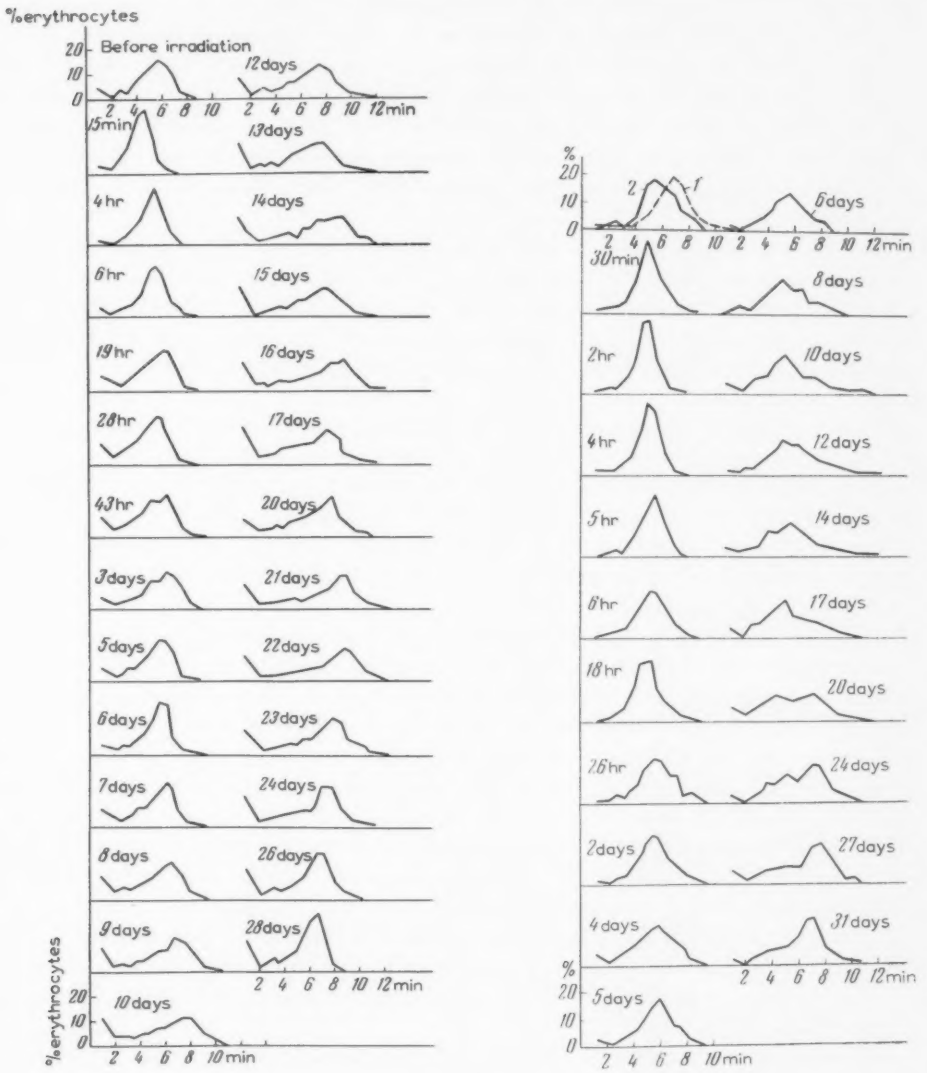

FIG. 4. Erythrogram: (a)—rabbit L-1-3 (400 r) and (b)—rabbit L-3-2 (800 r). (1)—before irradiation; (2)—15 min after irradiation.

based on photoelectric diffractometry, as to the heterogeneity of the erythrocytes in radiation sickness [10, 11] and the investigations of the resistance of the red corpuscles to photolysis carried out by Mochalina [5].

The results presented show that on exposure of the organism to penetrating radiation changes arise in the physico-chemical constitution of the erythrocytes, and develop further during the course of the irradiation sickness. This is characteristic of the red corpuscles as a whole.

Considerable interest attaches to the possibility of following the fate of individual qualitatively distinct groups of erythrocytes in the complex phenomenon of the radiation syndrome, and this can be carried out on the basis of differentiating the erythrocytes by their haemolytic stability. For this purpose it is more suitable to represent the results of the determination not in the form of percentage haemolysis curves, but in the form of differential distribution curves of the erythrocytes according to stability, thus putting into relief the qualitative composition of the erythrocyte population.

In Figs. 4a and 4b are represented the typical changes in the composition of the erythrocytes observed in the experiments. From these diagrams are seen the regular changes in the erythrograms of rabbits L–1–3 (400 r) and L–3–2 (800 r) during the period of observation. The erythrogram of the control rabbits remained practically unchanged during this period, and coincided with the initial erythrograms of the experimental animals.

During the first hours after irradiation a movement of the distribution maximum takes place in the direction of lowered stability. The maximum later returns to its initial position, and then a rise and growth of the right-hand side of the erythrogram begins until a new maximum is formed. In the erythrogram of rabbit L–3–2, which received 800 r, the interrelations of the old and new maxima are particularly clearly seen (Fig. 4b). The erythrogram of this rabbit from the twelfth day on stretches to the right and the maximum is smeared out. On the seventeenth day it can be noticed that on the background of the right branch a new maximum begins to form. On the twentieth day the old maximum is continuing its diminution, and the rising new maximum is increasing in height, and the erythrogram presents a characteristic two-peaked form. On the twenty-fourth day the new maximum exceeds the old, and on the twenty-seventh day alone remains; of the old maximum there remains a hardly noticeable rise. On the thirtieth day the maximum, formed by erythrocytes of raised stability, exhibits a tendency to shift into the position of normal stability. The transition of the maximum from raised stability to a normal position during recovery is well exhibited by rabbit L–1–3, which received the smaller dose of 400 r (Fig. 4a from twenty-second to twenty-eighth days).

Comparison of the dynamics of the changes of the erythrogram reveals that the changes taking place in the erythrocytes agree with the idea of a gradual shift in the stability of the erythrocytes (Fig. 4). This shift expresses a physiological ageing of the erythrocytes.

Erythrocytes of approximately the same stability can be conveniently grouped together. By considering the quantitative relationships between these groups it is possible to discern the laws of the changes in the qualitative composition of the red corpuscles during the development of the reaction to irradiation.

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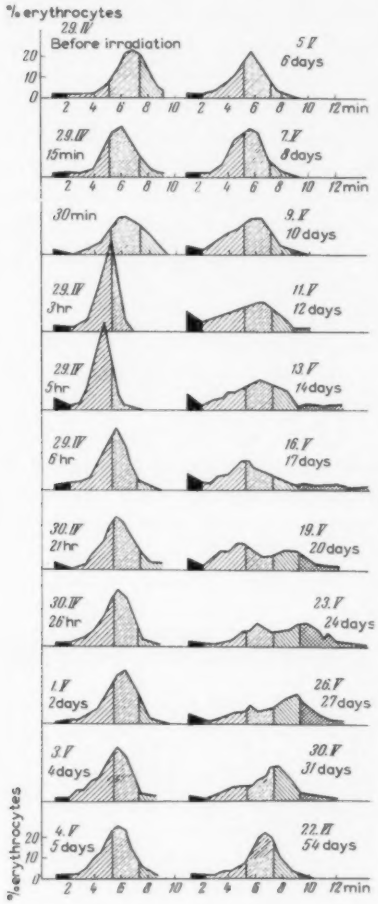

FIG. 5. Erythrogram of rabbit L–3–1 (800 r). From right to left: group of highly stable erythrocytes not found in the blood of the normal rabbit; group of raised stability; group of medium stability; group of lowered stability; pre-haemolysis changes and low stability erythrocytes.

Fig. 5 shows a series of erythrograms from rabbit L–3–1 (800 r). The erythrocytes are here distributed according to their stability into five groups, of which the three middle groups are inherent in normal blood, and the two extreme groups are pathological. The normal groups are: (1) from 2 to 5 min—erythrocytes of lowered stability, or, in the physiological sense, ageing erythrocytes; (2) from 5·5 to 7 min—erythrocytes of medium stability, mature erythrocytes; (3) from 7·5 to 9 min—erythrocytes of raised stability, young erythrocytes.

During extensive regeneration there appear erythrocytes of stability greater than 9 min constituting a group of highly resistant very young erythrocytes that ripen in the blood stream and are normally not elaborated in the bone marrow.

In the normal animal erythrocytes of less than 2 min stability are not found with certainty; this portion of the erythrogram corresponds to pre-haemolytic spherulation. During raised breakdown, erythrocytes of such low stability do appear, and their number is determined by a raised level of spherulation changes; they are therefore not included in the 100 per cent in calculating the erythrogram.

The quantitative side of the changes observed is represented by the diagrams shown in Fig. 6 (rabbit L–3–1) and Fig. 7 (rabbit L–1–2) with the same notation as in the erythrograms of Fig. 5. Under the diagrams are set out curves of the changes of the number of erythrocytes and of haemoglobin content in these animals, on the days covered by the diagram.

The dynamics of the changes in percentage distribution of the erythrocytes in groups of stability may be divided into phases differing in the nature and direction of the physiological processes.

In the first place there is a characteristic displacement of the whole erythrogram to the left; there is, therefore, a loss of stability throughout the whole mass of erythrocytes. There is a shift of the erythrocytes from one stability group to a lower one.

From a consideration of the erythrograms in Fig. 5 and the diagrams of Figs. 6 and 7, it can be seen that the degree of loss of stability is not identical for all the erythrocytes, and depends, apparently, on the original value of the stability. Thus

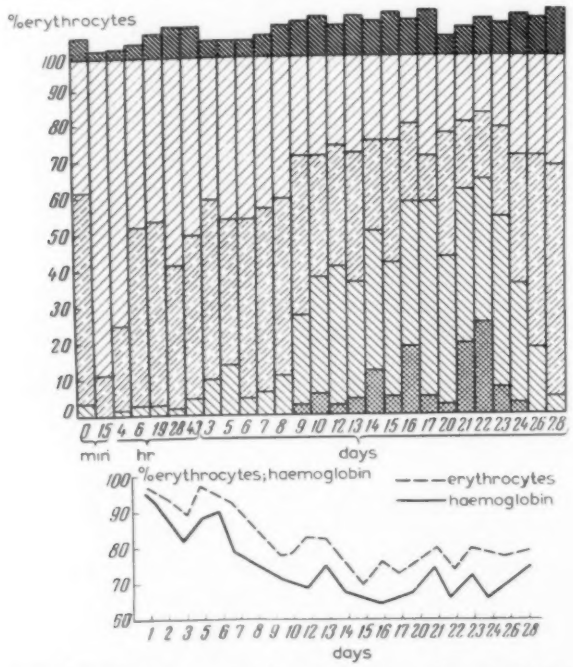

FIG. 6. Changes of qualitative composition of red corpuscles of rabbit L–3–1 (800 r)
Cf. explanation to Fig. 5.

erythrocytes of raised stability become moderately stable. This process is so intense that in rabbit L–3–1 after only 3 hr the group of erythrocytes of raised stability falls to zero. Further, it is possible to trace a continuance of the fall of stability of these erythrocytes, and some, at least, of them, passing through a condition of medium stability, arrive at a state of reduced stability, which is seen from the diagrams for the animal L–3–1. The initial number of erythrocytes of raised stability was in this case 40 per cent. After 5 hr there were none at all of them but the erythrocytes of medium stability constituted at that time 9 per cent; consequently not less than 31 per cent of the erythrocytes that had been of raised stability had gone into a condition of reduced stability.

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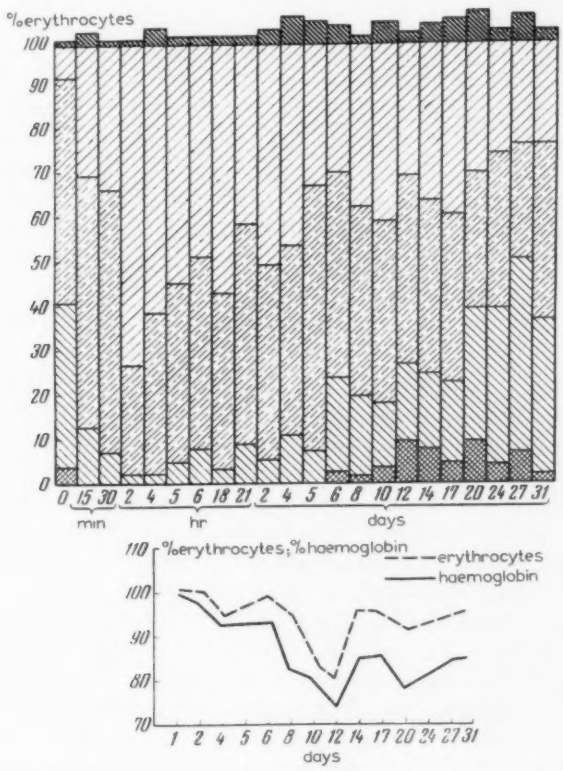

FIG. 7. Changes of qualitative composition of blood of rabbit L–1–2 (400 r). Cf. explanation to Fig. 5.

The erythrocytes of medium stability also suffer a lowering of stability; but it is not so considerable as in the erythrocytes of raised stability. It is very probable that this process can embrace the whole group of erythrocytes that were of medium stability, and the small number (9 per cent) of erythrocytes of medium stability on the diagram L–3–1 at the fifth hour evidently consists of erythrocytes developing from the condition of raised stability.

The stability of the erythrocytes of low stability is diminished least; this is evident from the fact that no significant growth of the group of low stability is noticed in this phase. Even if the stability of the erythrocytes of low stability is lowered still further this is not a large effect, and does not overstep the left boundary of the curve for this group. As a consequence of the observed gradation in the sensitivity of the erythrocytes to radiation, there occurs an accumulation of erythrocytes in the lowered stability group at the expense of the disappearance of those of raised stability and an impoverishment of the moderately stable erythrocytes. This is expressed on the erythrogram by a displacement of the maximum in the direction of lowered stability, and an increase in its size (Figs. 6 and 7, L–3–1, third–fifth hours). The diagrams give the quantitative aspect of the process at a glance.

A second phase replaces the first and is opposite in the direction of its changes of stability.

As was noticed above in the haemolysis curves, the second phase is characterized by a tendency towards normal in the distribution of the erythrocytes according to stability after this had been disturbed in the first phase. A study of the erythrogram according to stability groups permits the addition of a series of essential details to this general idea.

The process of restoration of stability does not affect all the erythrocytes simultaneously or to an equal degree. In animal L–3–1 at the sixth hour a considerable fraction of the erythrocytes (40 per cent) returns to a condition of medium stability, and the maximum of the erythrogram returns correspondingly to the values of the medium group. The number of erythrocytes of medium stability reaches the initial level (50 per cent). However the restoration of the raised stability of the erythrocytes does not occur at this time. At the twenty-first to twenty-sixth hours, and right up to the sixth day, the group of medium stability continues to be restored, and inside the group a displacement of the maximum in the direction of the normal position goes on. Quantitatively the group of medium stability on the second–sixth day even somewhat exceeds the initial level. The lowered-stability group remains quantitatively increased as compared with the background. The group of raised stability is extremely sparse and right up to the sixth day shows no tendency to increase. In its duration the second phase coincides with the clinical period of "pseudo-well-being".

The special features described for the course of the second phase permit the deduction that the restoration of the condition of the erythrocytes goes only as far as the condition of medium stability, and a return from medium to raised stability does not take place. The surpassing by the medium stability group of its initial level is explained by its containing erythrocytes that were of raised stability before the irradiation. The existence, in these days of the sickness, of an inconsiderable and not increasing group of raised stability is probably to be explained by the throwing out from the bone marrow of a small portion of rapidly matured cells, an effect which has been repeatedly described in radiobiological literature [4, 6, 7].

The second phase can therefore be called restorative only so far as the direction of the process is concerned, but not in its results. The quite limited reversibility of the damage to the erythrocytes is determined by the maintenance of an excess of the group of lowered stability and an irreversible loss of the raised stability group. The accelerated breakdown of the irradiated erythrocytes that appears in the third phase also confirms the incompleteness of their restoration.

The differentiation of the erythrocytes with respect to sensitivity that appears in the first phase is confirmed by the second phase: the more stable erythrocytes suffer a greater fall of stability and are restored to a lesser degree. If we regard the distribution of the erythrocytes by stability as an expression of their age-distribution, then the peculiarity observed confirms for the erythrocytes the general rule in radiobiology that damage by radiation is less in proportion to the maturity of the cells and tissues. It is of interest to note that in this case the rule is confirmed on non-nucleated cells.

The reasons for the variation of stability in the first and second phases require further investigation. On the basis of the results obtained, it can be stated that these changes do not arise as a result of a change of the constitution of the erythrocyte population. Sharp changes in the erythrogram develop in the first hours and even in the first minutes after irradiation (results on rabbit L–3–1). It is evident that in this period of time an appreciable change in the composition of the erythrocyte population cannot have taken place. In the course of the following 5–6 days, during the development of the second phase, regeneration is known to be suppressed, consequently an influx of erythrocytes does not occur. The absence of a sharp fall in the number of erythrocytes during this period (Fig. 6, results on L–3–1) shows that even the destruction of erythrocytes does not materially exceed the normal. Thus the variations of stability considered in the first and second phases arise in the same erythrocyte population as was present in the circulation before the experiment, and was subjected to irradiation.

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The mechanism of maturing of the erythrocytes can hardly be attributed to a direct action of the radiation, although this cannot be completely excluded. Indirect evidence against a direct action is given by the high resistance to radiation shown by erythrocytes outside the organism. In particular, the results obtained agree with the concept developed by Tarusov [12] as to the existence in the irradiated organism of haemolysins which probably are the cause of the early fall in stability of the erythrocytes. It is possible that the phasic changes of stability could be used for the investigation of the laws of formation and action of haemolysins.

With the beginning of the growth of a group of erythrocytes of raised stability the process enters a third phase, which coincides with the sixth to eighth day from the beginning of the experiment.

After the incipient rise in the blood of the number of erythrocytes of increased stability there appear highly stable erythrocytes, characteristic of this phase and not found in the normal. The erythrogram extends itself to the right, and its right wing rises. Later both these groups continue to increase, attaining in the animal L–3–1 at the twenty-fourth day 60 per cent (of which 35 per cent are highly stable and 25 per cent are of raised stability). On the erythrogram a second maximum is formed in the region of high stability. The appearance of highly stable erythrocytes is evidence of a recovery of regeneration. With the increased intensity of regeneration there are discharged into the blood stream erythrocytes of a lower degree of maturity than the erythrocytes usually elaborated by the bone marrow—they constitute the group of highly stable erythrocytes. It is possible that into this group there fall products of pathological regeneration (macrocytes, megalocytes, etc.); their differentiation by the erythrogram requires further investigation.

In spite of raised productivity of the bone marrow, anaemia develops at this time. As shown in Figs. 6 and 7 the number of erythrocytes and the haemoglobin content fall sharply in this phase. Consequently at this stage together with regeneration a breakdown takes place that exceeds quantitatively the erythropoiesis.

The group of erythrocytes of lowered stability decreases since the breakdown occurs at the expense of this group which comprises the physiologically most senile erythrocytes, and together with this group of cells of medium stability also diminishes by supplementing the diminishing group of lowered stability.

From the coincidence of an extensive breakdown with intensive regeneration it results that, at about the end of the phase under consideration, the erythrocytes that were in the blood at the time of irradiation are completely or in great part (depending on the dose) destroyed and replaced by new. The supposition that all the erythrocytes present in the blood stream are damaged at the moment of irradiation, and that there is an accelerated replacement of the blood during the development of radiation sickness can be reinforced by the following considerations. If the number of erythrocytes remains unchanged the loss (L) of erythrocytes from the blood stream must be equal to the gain (G) over the same period. But since during the phase under consideration there is a fall in the number of erythrocytes (ΔE) with a raised level of gain and loss, $L+\Delta L-G-\Delta G=\Delta E$ where ΔL is the excess loss above normal and ΔG is the excess gain above normal. Regarding L and G as the physiological production and destruction of erythrocytes, and equating them, we have $\Delta L=\Delta E+\Delta G$. For example, for animal L–3–1 a noticeable regeneration begins on the sixth day and rises to its maximum on the twenty-fourth day. During this time $\Delta E = 30$ per cent, $\Delta G = 38$ per cent ($54\times 0{\cdot}7$ taking into account the decrease in the total number of erythrocytes). Hence $\Delta L = 68$ per cent.

Consequently, during the period from the sixth to the twenty-fourth day, over and above the normal replacement of the red corpuscles, 68 per cent of the erythrocytes disintegrated. It is possible that this number is a little less than the true one, since we have not taken into account that the erythrocytes produced at this stage of the disease mature and age more rapidly than normal, and therefore their transfer from one stability group to another, and their replacement, go on more quickly.

Considering a normal replacement of the blood and a heightened disintegration of the erythrocytes after irradiation, it can be supposed that under the conditions of our experiments between the twenty-fifth and thirty-fifth days all the erythrocytes present in the organism at the moment of irradiation are replaced. The replacement of the blood can be also followed directly from the development of the maxima of the erythrogram, particularly clearly shown with large doses (Fig. 5).

From this exposition it follows that the concept of a sluggishness of regeneration in radiation sickness cannot be extended to the third phase (at least in non-lethal cases). In this phase the regeneration is quite extensive, but with all its intensity it is not capable of compensating the still more intense destruction which, in the long run, leads to an anaemia which masks the regeneration.

With the subsidence of the regeneration, the process goes over into the fourth phase (twentieth to thirtieth day). In this phase the group of highly stable erythrocytes ceases to expand and gradually disappears. Correspondingly the number of cells of raised stability falls to normal and sometimes even below. Maturation

predominates over production. As a result, the group of moderate stability increases in size. The qualitative composition of the red corpuscles becomes normal.

With large doses, equilibrium usually arises at a lowered level of erythrocyte content. This may be explained by a functional exhaustion of the bone marrow after the very extensive regeneration vitally necessary in the period of increased breakdown of the irradiated blood, and occurring during conditions of radiation damage to the erythropoietic tissue.

To the end of the experiment, particularly with large doses, the qualitative composition of the red corpuscles does not become completely normal. This shows the prolonged after-effect of irradiation on the erythropoietic system.

The phases of change in the qualitative composition of the red corpuscles, as shown on the erythrograms, coincide in general features with the generally accepted clinical division of radiation sickness into four periods. From this follows a possibility of using erythrograms in practice for the diagnosis and prognosis of radiation sickness.

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The first phase of change of the erythrograms corresponds to the period of initial reactions. The depth and incomplete reversibility of the changes in the erythrocytes in this period may serve as an indication of radiation damage. It is particularly important that the changes appear in the early stages. From two erythrograms obtained a small interval of time apart it is possible to judge of the direction of the development of the process (development of the first phase or transition to the second). It is still early to speak of a strict quantitative dependence of the depth of changes arising in the erythrogram on the degree of damage, since in this phase unspecific shock-symptoms are superimposed on the effects of the radiation, the more so as the experiment was carried out on rabbits, which are particularly susceptible to shock.

In the latent period (pseudo-well-being) which is unmistakeably expressed by the second phase of the erythrograms, the change of condition of the erythrocytes continues to develop. The shock symptoms largely disappear, and it is possible to judge the radiation damage by the changes persisting in the erythrogram. The erythrograms in this phase express, as it were, the physiological ageing of the blood. The series of erythrograms obtained in this period show the direction of the development of the process.

The transition of the changes in the erythrograms to the third phase is evidence of the beginning of a revival of regeneration. The intensity of regeneration can be measured by the increase of the group of cells of raised stability, and the appearance and changes of the group of highly stable erythrocytes.

The level of breakdown of the erythrocytes at this stage can be determined from a measurement of the number of erythrocytes, in conjunction with the erythrograms. Some idea of the increased breakdown and degenerative changes in the erythrocytes is given by the increase of the low-stability group.

Erythrograms of the fourth phase correspond to the period of resolution of the disease. They permit a characterization of the dynamics of normalization of the composition of the red corpuscles, and of the return to the initial condition or the presence of permanent residual changes.

Thus the taking of the erythrograms during the course of the disease gives a picture of the dynamics of the changes in condition, and of the replacement of the

composition of the red corpuscles during radiation sickness. The possibility of mass analysis, and the objectivity of the method, recommend the erythrogram as one of the means of investigation of radiation disease.

CONCLUSIONS

(1) The qualitative composition of the red corpuscles, expressed by the distribution of the erythrocytes according to stability, shows regular changes during the development of the radiation syndrome, and these can be divided into four phases on the basis of the corresponding periods of the clinical disease.

(2) A latent period in the reaction of the red corpuscles to irradiation is not seen. The change begins from the moment of the irradiation and develops continuously (without a period of pseudo-well-being) right up to the termination of the disease.

(3) In the first and second phases the damaged erythrocytes show physicochemical changes which are reflected in a leftwards displacement of the erythrogram. A sharp displacement of the erythrogram to the left continuing in the second phase must be regarded as an unfavourable sign, indicating severe damage to the erythrocytes and a condition of shock.

(4) The changes in qualitative composition of the red corpuscles show that at doses of 800–900 r all the erythrocytes present in the blood stream are affected. The degree of damage varies, and depends, apparently, on the age and condition of the erythrocyte at the moment of irradiation.

(5) In conjunction with a determination of the number of erythrocytes, the erythrograms express quantitatively the dynamics of the breakdown of the damaged erythrocytes and the replacement of the blood during radiation sickness.

(6) The third and fourth phases express the processes of regeneration, replacement of damaged erythrocytes, and normalization of the composition of the red corpuscles. An extension and rise of the right branch of the erythrogram in the third phase is a sign of the beginning of regeneration. The number of erythrocytes of raised and high stability marks the extent of regeneration.

(7) The regularity of the changes in the erythrogram suggest their future use for the detection and definition of the course of acute radiation damage.

Translated by T. R. PARSONS

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BIOPHYSICS AND ITS PLACE IN MEDICAL EDUCATION*

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VOL. 2 1957

(*Received* 4 *October* 1956)

ONE of the most important tasks of higher medical education is the formation in the future doctor of a correct materialistic attitude. This task must be reflected in the content of the curriculum, which is devised by taking into account the dialectical interdependence of the branches of science and their materialistic nature.

The materialistic nature of the biological and medical sciences consists in this, that behind the exceptionally complex phenomena of the vital activity of the organism lie the general laws of physical and chemical phenomena. Analysing the question of the types of motion, F. Engels pointed out that life—the organic type of motion—was developed on the basis of simple types of motion: physical and chemical. Correspondingly, those sciences which study the organic type of motion are developed on the basis of physics and chemistry. However, the latter at the same time have this qualitative distinction: they are more highly developed. Thus, for example, when defining physiology as a science, Engels writes: "Physiology is, obviously, the physics and especially the chemistry of the living body, but also it is not just a speciality of chemistry: on the one hand its sphere of activity is organic and on the other hand it is, together with this, raised to some higher level" ("Dialectics of Nature" (Russian), p. 204, 1953).

Thus the physics and chemistry "of the living body" are distinguished from the usual physics and chemistry, which chiefly study the laws of inanimate nature. In accordance with the historical development of the science of the internal media of the organism from chemistry, the "chemistry of the living body", or biochemistry, has split off and has occupied a definite place in the overall plan of medical education.

Then, as the application of physical methods and concepts was developed for the study of the vital processes of organisms and especially processes of interaction of the organism with the external environment, the independent discipline of "the physics of the living body", or biophysics, began to take place. Problems of biophysics have a special significance for medicine at the present time in connexion with the materialist philosophical teaching of I. P. Pavlov concerning the most complex processes of vital activity of the organism, the equilibration of the organism with the external environment.

* Biofizika **2**: No. 4, 536–539, 1957 [Reprint Order No. BIO 70].

In spite of this, the problems of biophysics are very poorly reflected in medical education and biophysics as a discipline is absent from the curricula of medical institutes. (An exception is the Kirov Military-Medicine Academy, in the curriculum of the first course of which is the subject "medical physics", embracing biophysical problems in some measure.)

There is at the present time in the curricula of medical institutes only a course of physics which is read in the first and second semesters. Until recently it was considered as a general educational subject supplementing and deepening the physics course of the secondary school. In 1955 the programme of this course was somewhat revised and it was given a practical direction towards a study of the physical bases of the construction of medical (therapeutic and diagnostic) instruments which were most widespread in practice.

The inadequacy of this course is due to two fundamental causes. In the first place the time spent on it is insufficient, and so is the number of lectures (68 lecture and 68 laboratory hr). In such a time it is scarcely possible to include the necessary prerequisites of general physics and give some fundamentals of the construction of the most important medical and hygiene instruments and apparatus. In a course of such length it is impossible either to expand into the biophysical field or to introduce into it new, modern methods of biophysical investigation which are applied in laboratories and in the clinic.

Even the course of medical physics which is given in the military-medicine academies has approximately one and a half times more lecture hours.

The second inadequacy of the course is that it occurs in the curriculum in the first year of instruction when the students have only just started to study medical sciences (anatomy and general biology) and still cannot understand the special direction of the physics course. Moreover, even with those few facts on the theory of the structure of the atom and nucleus, the quantum nature of light, etc., and also on the fundamentals of the construction of special medical apparatus (electrocardiograph, the simplest physiotherapy equipment, etc.) which one succeeds in including in this course, it is just the same as with the old course: when they are most necessary for the students they are simply forgotten.

At the same time, the necessary elements of biophysics are dispersed among many specialist disciplines. Especially many elements of biophysics occur in the course of normal physiology, for which they are a necessary introduction to a whole series of problems. The elements of biophysics also form the content of the introductory chapters of many other disciplines: functional diagnostics, physiotherapy, roentgenology, radiology, a number of the hygiene sciences and so on. Because of this dispersion into small problems, the significance of biophysics, as a science concerned with the laws of the materialistic (physico-chemical) basis of physiology and the specialist medical sciences, disappears. On the other hand, in practice, often during the exposition of a specialist discipline these introductory biophysical chapters, because of limitations of space, are simply omitted. A graphic example is the evolution of the textbook on normal physiology by K. M. Bykov. In the 1950 edition almost every chapter contained the biophysical prerequisites (although printed small). In the 1955 edition, despite the book's overall increase in size, most of these prerequisites were not stated in the book.

We will have a look at how biophysics is taught to students and doctors beyond our borders. We do not directly possess the facts about the curricula, but we can judge the situation from the literature. Excluding from consideration the vast periodical literature, one can note the existence of a number of specialist manuals on biophysics for students and doctors: for example, the works of Stuhlman [1], Burns [2] and others. These text-books are similar in nature to Efimov's book [3]—our single published text-book on medical biophysics. For text-books of a similar kind it is possible to refer also to Ernst's book [4], published in Budapest in 1947.

Beier and Dörner [5] have just published a course of physics for students and doctors, on its applications to biology and medicine; judging by the first volume coming from the press, in this text-book the facts of biophysics are successfully united with the necessary prerequisites from general physics.

Finally, a considerable number of manuals under various names should be noted; these cover, chiefly, problems of physiological and biophysical research methods, applied to medicine. Such manuals are, for example, Glasser [6], "Biophysical Research Methods" [7], "Advances in Biological and Medical Physics" [8] and others, and also a similar work edited by Uber [9], translated into Russian.

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Hence we may conclude that, beyond our borders, a great deal of attention is paid to problems of medical biophysics, physics and biophysical research methods in medical education. All this forms a basis for raising the question of a thorough revision of the time, place and content of a physics course in the curricula of medical institutes.

What kind of tasks must be set before a new course? First, we must give a general education on the materialistic (physico-chemical) basis of the laws studied in the specialist disciplines. Second, we must give the fundamentals of "medical biophysics", that is, the particular laws of biophysics which are specific for these and other branches of medicine. Third, we must give the fundamental of biophysical methods of research and practice, applied in the clinic, and also the fundamentals of the construction of the corresponding apparatus.

Hence follows the answer to the question of duration, place and content of the course. The duration should be increased to at least twice its present length. The place should be among the specialities and not among the general-educational disciplines, i.e. in the later courses.

As for the detailed content of the proposed course, in this article we are not setting ourselves the task of giving a working programme, this is the business of the appropriate bodies, but we think it expedient to have a general sketch of the contents in view. Such a course must, as has been indicated, contain, in the first place, the fundamentals of medical biophysics, in the second place the fundamentals of biophysical methods of research and practice, in the third place some branches of general physics, supplementing in whatever way necessary the secondary school course (in particular that branch devoted to the structure of matter and especially nuclear physics).

The fundamentals of medical biophysics, in our opinion, should contain the following sections: kinematics and dynamics to the extent used in analysing the function of the muscle–skeleton system, the physical laws of the circulation in the blood system (the physics of the movement of a viscous liquid along tubes with elastic

walls); the energy balance of an organism (the application of the elements of thermodynamics to the living organism); and the physical bases of the temperature regulation of an organism (thermal conductivity, convection, radiation and evaporation).

A suitable place in the course on the fundamentals of medical biophysics must be found for the physics of the structure of the sense organs (the ear and the eye). A study should be made of the optics of the eye, supplementing the eye with optical instruments, light measurements connected with the light sensitivity of the eye (the concept of "brightness" and so on). Similarly, in the field of acoustics, the ear as an acoustical instrument, the fundamentals of audiometry (the concepts of loudness and the decibel scale) and so on should all be studied. It is appropriate to stress here that all these problems do not enter into a physiology course and are inadequately explained, although instruction on analysers occupies an essential place. Physiology discusses the analyser as a whole, especially the connexion of its peripheral end, i.e. the sense organ, with the central nervous system. Biophysics analyses the structure of the peripheral organ itself, and also the conditions of the conversion of the energy of an external stimulus into the nerve impulse.

To medical biophysics also belongs that range of problems connected with the appearance of electrical phenomena in biological media: mobility of ions, phenomena at the boundary of two media, polarization of semipermeable membranes,convection currents, electro-osmosis and so on.

There is another very wide range of problems pertaining to medical biophysics which are concerned with the mechanism of the primary action on an organism of various external physical influences or so-called agents—mechanical waves (vibrations), heat and cold, air pressure and rarefaction, ultrasonic waves, and especially various types of electric current and electromagnetic waves, applied in physiotherapy, including light and other types of electromagnetic radiation.

In its vital processes the organism reacts to external stimuli by various physical agents which act on our analysers or directly on one or other part of the body. The biological reaction of the organism to an external agent is a complex process consisting of a series of successively developing links. The first link is the physical process, the primary action of the physical agent. The problems concerned with the primary action of physical agents, constitute an appreciable part of biophysics.

In this section one should include the problem of the physical bases of the various methods of physiotherapy. Of special importance in modern conditions is the primary action on the organism of ionizing radiations and the associated problems of measurement and dosimetry of these radiations.

Finally, the last section of medical biophysics—the section soonest applied—must embrace a considerable range of modern methods of biophysical research in these or other processes in the organism: the determination of viscosity and surface tension of biological fluids, tissue thermometry, chronaximetry, the determination of lability, the investigation of bioelectric potentials, special optical microscopy (fluorescent, phase-contrast, polarization, etc.), electron microscopy and so on.

CONCLUSIONS

(1) In order to teach future doctors a materialistic outlook and in order to provide the necessary information in the field of the physico-chemical bases of vital phenomena,

and also so that they know the possibilities of the practical utilization of the attainments of the modern techniques of biophysical research, diagnostic methods and therapeutic procedures, it is essential to revise the time, place and content of the physics course in higher medical schools.

(2) It is necessary to increase the duration of the course, to transfer it to a later semester and to give it a biophysical slant: the course must contain, besides the necessary prerequisites of general physics, problems of medical biophysics, the fundamentals of biophysical methods of research in experiments and in the clinic, and also the principles of the construction of modern diagnostic and therapeutic apparatus.

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REPORT

FIRST INTER-INSTITUTE CONFERENCE ON RADIOBIOLOGY*

FROM 25 to 28 February 1957 there gathered in the Department of Soil Biology of the Moscow State University the first All-Union Inter-Institute Conference on the problem of the "Biochemical and physico-chemical bases of the biological action of radiations". About 800 delegates from various institutes and organizations of the Soviet Union, including representatives of 32 higher educational institutions of our country, together with foreign guests, took part in the conference, which was called by the Ministry of Higher Education of the U.S.S.R. and the Moscow State University.

In his introductory remarks Professor B. N. Tarusov, Head of the Department of Biophysics of the Moscow State University, described the general state and prospects of further development of the biophysics of radiation.

All the growing application of atomic energy in science, technology and the various branches of national husbandry, has led to a sharp increase of interest in the biological action of ionizing radiations. The task of the conference was to direct the attention of the outlying institutes to investigations in radiation biology by means of extensive scientific information and co-ordination of research.

* Biofizika **2:** No. 4, 540–544, 1957 [Reprint Order No. BIO 71].

The present-day state of the biophysics radiation may be described as follows. The fixing of the range of applicability of the target theory (viruses, structures concerned with heredity) has guided investigations of the mechanism of the primary action into the direction of the study of the formation of biologically very highly active radicals from the aqueous phase. Neither the target theory nor the theory of indirect action explains the mechanism of reinforcement of the radiobiological effect. The hypotheses that have been put forward with this object by radiobiologists (Ellinger's theory of histamine poisoning, and the theory of enzyme inactivation by Barrow and others) do not accord with the facts. A series of other conjectures (destruction of adaptive complexes, disharmony of development of irradiated cells and others) pass over in silence the question as to the nature of the processes that follow the initial physico-chemical changes accompanying the absorption of quanta of ionizing radiation, and calling forth a variety of biological response. There arises the problem of the mechanism of the reinforcement of the initial chemical effects of the radiation to the stage of radiation sickness starting from an analysis of the physico-chemical regularities. Tarusov suggests that such a mechanism could be constituted by the generation and development of branched chain reactions, supposedly in lipoproteins, with a resulting destruction of most important micro-structures and the appearance of various toxic products. This is followed by the stage of frank radiation sickness.

For the elaboration of a theory of the mechanism of radiation after action the study of the kinetics of the post-irradiation changes are of first-rate importance on both biochemical experiments *in vitro* and in living systems.

Detailed theoretical papers were read by Prof. A. M. Kuzin on biochemical problems of radiation, Prof. M. N. Meisel on the action of ionizing radiation in various cell structures, and by N. P. Dubinin, corresponding member of Academy of Sciences of the U.S.S.R., on the subject "Ionizing radiation and heredity".

Prof. A. N. Kuzin (Institute of Biophysics, Academy of Sciences of the U.S.S.R.) dwelt in his speech on the basic problems with which the biochemistry of radiation is confronted.

The profound changes arising in organisms after exposure to ionizing radiations are the consequences of initial destruction in various biochemical components. The initial links in these destructive processes are made up of the formation of activated molecules, ions and free radicals. It is very important for the development of a general biological theory of the action of radiations to study the nature of the activations of the substances in submicroscopic structures composed of macro-molecular polymers, and to investigate the quasi-stable conditions and chain reactions.

An investigation is essential of the changes in the submicroscopic structures of cell organelles containing nucleoproteins, nucleic acid and glucoproteins—substances possessing a high sensitivity to radiation, and playing an important part in the processes of cell division, in the transmission of hereditary characters, in the permeability of tissues, and in other functions of the cell easily affected by radiation.

The destruction of the normal structure of biological polymers, and the distortion of the processes of material metabolism lead to the appearance of toxic products in the organism. Radiotoxins arise at various stages of radiation sickness, and have different natures and modes of action. To deal with the toxological problems of

radiation sickness together with the establishment of the part played by destruction of endocrine regulation of metabolic processes is an immediate task of radiation biochemistry.

Investigations in the field of radiation biochemistry must furnish the basis for the rational search for new methods of diagnosis and treatment of radiation sickness, and for a wide utilization of ionizing radiations in medicine, agriculture and the food industry.

Prof. M. N. Meisel (Institute of Microbiology, Academy of Sciences of the U.S.S.R.) presented to the members of the conference the results of a study carried out in his laboratory of the action of radiations on various components of the cells of micro-organisms and of bone marrow, and also of the functional peculiarities of irradiated cell populations and their immediate descendants. With the help of the newest methods of microscopy it was possible to show that ionizing radiation exerts an influence on all cell structures without exception, although different parts of the cell differ very widely in sensitivity. The most vulnerable are the mitochondriosome apparatus and the nucleoprotein complexes—structures responsible for the metabolic and reproductive activity of the cell. There are large differences between the sensitivities of the different enzyme systems which determine the metabolic processes of the cell and its offspring. The most sensitive function is reproduction, the least sensitive are growth and respiration. By tracer techniques, and by spectral and biochemical methods of investigation, there has been shown a difference between irradiated and non-irradiated yeast cells in their content of free amino acids and sulphydryl groups, an increase in the content of free methionine, and an excretion of phosphorus and of nuclestides. In irradiated yeast cells there is increased biosynthesis of ergosterol and a suppression of the synthesis of pantothemic acid, these changes being retained through several generations. At the end of the paper a colour film entitled "Action of radiations on micro-organisms" was shown.

N. P. Dubinin, corresponding member of the Academy of Sciences of the U.S.S.R., presented a communication on fundamental questions of radiation genetics. The action of ionizing radiations is characterized by a direct proportionality between mutations and dose, an independence of frequency of mutation of the intensity of the radiation, and an absence of threshold doses in the action of ionizing radiations on hereditary structures. The last circumstance compels us to approach with all strictness the calculation of maximum permissible doses suggested by certain foreign investigators, by whom the problem of permissible level of radiation is shown from its scientific foundations for the purpose of justifying the appearance on the globe of zones of raised radioactivity. If the action of any doses of ionizing radiation on man is unconditionally genetically harmful we must strive to reduce the dose received. Nevertheless, in the selection of plant and micro-organisms, and in other branches of agriculture and biology, high-energy irradiations are of great value, serving as a factor for the production of radiation mutants, resistant to diseases and with changed chemical composition of the grain, new reactions to fertilizers, etc. The lecturer emphasized that the new branch of science—radiation genetics—requires the vocational training of fully competent personnel having a command of genetics, chemistry, physics and mathematics.

Considerable interest was aroused by the researches of a group of co-workers

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—E. V. Burlakova, W. G. Dzantiev, G. B. Sergiev and Prof. N. M. Emanuel'—of the Department of Chemical Kinetics, on the origin and development of radiation sickness. The large biological effect corresponding to small doses of radiation and the presence of a period of apparent well-being in the course of radiation sickness, are evidence in favour of the idea that radiation sickness is determined by the course of a chemical branched chain process. A series of facts (the oxygen effect, the part played by anti-oxidants) points to the process of oxidation as at least one of the important chain processes taking place in the irradiated organism. Burlakova *et al.* studied the kinetics of the chain radiolytic oxidation of fat of animal origin (fish oil) containing esters of unsaturated fatty acids. The kinetic curves are marked by the presence of a period of induction with a subsequent rapid increase in the quantity of the products of oxidation, depending on the dose of radiation and on the temperature. It is suggested that the action of the ionizing radiation is partly bound up with the destruction of natural oxidation inhibitors. This latter was shown in experiments on irradiation of 0·01–0·05% solutions of hydroquinine and butyloxyanisole in the methyl ester of oleic acid. At various stages of the oxidation the biological activity of the oxidized fat was investigated. It was established that the active principle is soluble in water. The greatest toxicity was observed at the stage of maximum acidity.

In the work of K. I. Zhuravlev (Institute of Biophysics, Academy of Sciences of the U.S.S.R.) interesting results were obtained in the study of the primary physico-chemical changes in irradiated fat extracted by ether from the liver of the ox. In the liver fat two types of chain reactions, not characteristic of non-irradiated fats, and developing only under the influence of radiation, were observed: (1) an unbranched chain reaction without an induction period, but with a high yield of ions; (2) a branched-chain reaction possessing an induction period. The reaction developed in a gradual curve and gave a rise and fall of the content of peroxides. The auto-catalytic reaction of anti-oxidation arising in liver fat independently of irradiation developed with some delay owing to competition from a reaction of the second type. The haemolytic factor appeared in liver fat together with an accumulation of free fatty acids. Results on the law governing the accumulation of acids, and a classification of the types of reaction forming peroxides in irradiated fats, were given for the first time.

A series of communications was devoted to the kinetics of the after-effect of radiation in living systems.

Seeing that the laws governing the development of damage in individual cells have been extraordinarily little studied, V. I. Korogodin (Department of Biophysics, Moscow State University) has undertaken an investigation of the kinetics of post-radiation changes in yeasts. The criteria of damage used were the dynamics of the first budding, the rate of formation of microcolonies and of the growth of macro-colonies. All the irradiated cells showed damage, but the damage had reversible and irreversible characteristics. Reversibly damaged cells formed normal macrocolonies, of which the average value of the square of the radius bore an inverse straight-line relationship to the dose. With the aid of the technique of fixed microcolonies, developed by the author, it proved possible to establish certain forms of death of the irradiated yeast cells. It turned out that cells inactivated in the region of mean lethal doses lose their reproductive capability only after effecting four to seven cycles of

multiplication. It may be supposed that, in spite of the prevailing opinion, the reason for the post-radiation death of the cell is not the stopping of reproduction, but an inactivation of the cell arising on attainment by the post-radiation changes of definite threshold values.

Peculiarities of the radiation after-effect reactions in Hydra formed the subject of a communication by G. G. Polikarpov of the Sevastopol Biological Station. The main attention of the investigation was turned to the relation between length of latent period and the dose of X- or γ-rays, temperature and oxygen tension. From a comparison of the various parameters (effect, dose, course of appearance of lesions, temperature coefficients, dependence of latent period of early and remote effects on hypoxia) a conclusion was drawn as to the difference of nature of the reactions determining the early and late waves of radiation sickness in Hydra.

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G. V. Sumarukov presented the results of a study made in the Department of Biophysics of Moscow State University of the dynamics of radiation sickness in grain weevils. The distribution curve of the relation of deaths of the insects to time after irradiation with doses of 500–6000 r of γ-rays corresponded to a Gaussian distribution, with a maximum on the tenth to eleventh day after irradiation, i.e. the ordinate of the point of maximum mortality proved not to be dependent on the dose. A lowering of oxygen tension at the time of irradiation acted in a manner analogous to a reduction of dose: there was simply a reduction in the percentage of insects affected. A temporary lowering of temperature after irradiation inhibited the development of the sickness. The curve of mortality with time turned out to be sharply displaced to the right, and was asymmetric. Thus the laws governing the latent period of radiation sickness have been established for adult insects.

V. A. Blinov (Molotov University) has found that, with growth, embryos of the pond snail become more resistant to penetrating radiation. Large doses of X-rays (95,000 r) stopped growth and caused death at the 4–8 blastomere stage.

I. A. Motuzova (Department of Biophysics, Moscow State University) presented the results of a study of the influence of the conditions of irradiation on the form of the survival curves of *Bacillus coli*. Variously dense suspensions in water and in broth, of the bacteria at various growth stages were irradiated with γ-rays. Independently of the conditions of irradiation, the survival curves for the bacterial suspensions in water were of exponential form, but in the case of suspensions in broth such a regularity was observed only on irradiation of the relatively dilute and younger cultures. The results obtained show that the form of the survival curve depends on the physiological condition of the culture, and is determined, apparently, by the peculiarities of the kinetic characteristics of those processes that arise in bacterial cells on irradiation under various conditions.

A detailed paper was read by Iu. A. Kriger (Department of Biophysics, Moscow State University) who carried out in collaboration with E. S. Elkhovska an investigation on the physico-chemical properties and structure of erythrocytes during the action of γ-rays. The authors were the first to attempt to carry out a parallel investigation of the electrochemical properties of irradiated and non-irradiated erythrocytes and of their electron-microscopic pictures. The result of the experiment showed that the first stage of change of resistance, measured by the method of low-frequency electrical conductivity, corresponded to the swelling of the erythrocytes with

preservation of the integrity of their physico-chemical structure. The second stage gave evidence, apparently, of a simultaneous increase of volume of the erythrocytes and the appearance in them of an insignificant ionic conductivity. The electron-microscopic investigations confirm the results of the conductometric measurements. Irradiation in itself may not destroy the submicroscopic structure of the erythrocyte. But physico-chemical changes taking place in the latter weaken the link between individual components to such an extent that the subsequent hypotonicity causes profound damage to the cell. The authors cast doubt on the correctness of Feil's theory. At the basis of radiation haemolysis there evidently lies a destruction of the lipoprotein complexes that determine the high electrical resistance of the erythrocytes.

A conspicuous place in the work of the conference was occupied by papers on the biochemical action of radiation, extending over problems of coagulability of the blood in experimental radiation sickness and questions of autolysis and haemolytic activity of irradiated tissues.

The many years of research carried out in the Department of Animal Biochemistry of Moscow State University under the leadership of Prof. B. A. Kudriashov has resulted in a knowledge of the mechanism of haemorrhagic phenomena in radiation sickness. It has been shown that the basic reason for the disturbance of the coagulability of the blood is an insufficiency of blood prothrombokinase arising in the irradiated animals. The lack of prothrombokinase leads to a diminution, or even complete cessation, of thrombokinase formation in the blood, in spite of the preservation in the plasma of thrombotropin, the protein that activates prothrombokinase.

The thromboplastic activity of the blood of white rats falls rapidly with increase of dose, and at 400 r reaches a minimum. At the same time the strength of the capillaries falls, and these two factors in combination produce conditions leading to the haemorrhagic symptoms.

Local screening of the region of the liver, and combination of zonal protection with administration of vitamin B_{12} and folic acid, partially preserves the organism against the lowering of the thromboplastic activity of the blood and the development of haemorrhages in the post-irradiation period. A method has been worked out for stopping bleeding into the parenchyma in radiation sickness using a solution of thrombin together with a plugging of the wounds by fibrin.

G. V. Andreenko and G. G. Bazaz'ian (Department of Biochemistry, Moscow State University) elucidated in their contributions more details of the questions of the action of vitamin B_{12} and of folic acid on the thromboplastic activity of the blood of normal and radiation-sick white rats, as touched on in Kudriashov's communication, and of the reasons for the haemorrhagic tendency in radiation sickness evoked by subcutaneous injection of radioactive phosphorus.

A paper by V. N. Benevolenskii (Institute of Biophysics, Academy of Medical Sciences of the U.S.S.R.) was devoted to a consideration of the mechanism of formation, in animals exposed to ionizing radiations, of the tissue haemolysins discovered by A. S. Molchalina (laboratory of B. N. Tarusov). The laws governing the action of the haemolytic factor *in vitro*, and the dynamics of accumulation of haemolysins in the irradiated organism, indicate a possible link between this factor and the chain reactions induced by the irradiation. In a number of particulars (thermostability, lack of species-specificity, effect of dilution, pH, etc.) the tissue haemolysin of

irradiated rats showed similarity to the haemolysins formed on autolysis of liver from the non-irradiated animal. This similarity was confirmed by electron-microscope analysis. The author suggests that the formation of tissue haemolysins in the irradiated organism is to some extent bound up with phenomena reminiscent of an autocatalytic process. The appearance of tissue haemolysins, both in the course of autolysis and in radiation sickness is at least partly connected with the activation of an enzymic complex evoking the breakdown of protein–lipoid components of the tissue. The haemolytic factor, apparently, is the end product of a chain reaction.

The haemolytic factor appears in the liver of animals not only on external irradiation, but also on internal administration to the organism of radioactive substances. L. L. Khamaide (Institute of Biophysics, Academy of Medical Sciences of the U.S.S.R.) has shown that the haemolytic activity of the liver of mice is developed considerably more quickly, and is marked by a higher level, on injecting a solution of the fission products of uranium then under the action of analogous doses of radiocaesium.

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In the paper by Prof. M. V. Kirzon and M. G. Pshennikova (Moscow State University) the results of an investigation into the action of X-rays on frog's nerve–muscle preparation was presented. In the parts neighbouring the irradiated part there develops an increased excitability. This process, arising during even the first minutes of the irradiation, continues to increase for the course of some hours after the irradiation. The authors regard it as possible to assume the existence of impulseless effects spreading through the interneuronal synapases of the central nervous system. The process that invades the nerve under the influence of impulseless effects arising from the irradiated region most probably does not coincide with a process of injury.

A. F. Ivanitsova (Institute of Animal Morphology, Academy of Sciences of the U.S.S.R.) presented a paper on the influence of ionizing radiations on the blood-forming organs. The course of post-irradiation changes was investigated by the method of tissue culture. The paper was accompanied by a cinemicrographic film.

The work of E. G. Lomovskaia and F. B. Shapiro touched on questions of protection against penetrating radiations of mammalian embryos at various stages of pregnancy. It was shown that damage by radiation of embryos of white mice was determined by the direct action of the radiation. The protective effect of heroin was clearly shown even on irradiation at the most sensitive period of embryogenesis (9–12 days pregnancy) and consists of a sharp lowering of mortality of the foetuses and frequency of malformations.

Papers devoted to the discussion of questions of the practical utilization of radiation in the national economy were presented to the Conference.

Universal attention was aroused by the investigations of S. I. Alikhanian (Institute of Antibiotics) who has succeeded in applying radiant energy in the selection of micro-organisms that produce antibiotics. By using super-high doses of ultra-violet rays in combination with photo-reactivation a new and more active form of streptomycin (strain 213) was separated. As a result of irradiation of the hybrid actinomycetes new strains of "New Hybrid" serial no. G-31 were isolated. High frequencies of mutations were obtained by the action of radiation on spores previously treated with non-mutagenic doses of ethyleneimine. Ultra-violet rays were used in the isolation of the highly active strain L S T-118 of terromycin. In experiments on irradiation

of spores of producers of albomycin a new highly active strain was obtained by X-ray selection. In conclusion Alikharian pointed out that without the use of short-wave radiation in the genetics and selection of micro-organisms it would be generally impossible to set up an extensive production of antibiotics.

Prof. F. M. Kuperman (Department of Darwinism, Moscow State University) presented to members of the Conference the results of his experiments, carried out in the 'thirties, on the action of ionizing radiations on variability in plants. V. A. Guseva (Lecturer, Gor'ki University) spoke on the effect of irradiation with ^{60}Co on the yield of agricultural crops—buckwheat, peas and potatoes. The plant reacts first of all as a single organism. Radiation affects all the functions of the plant organism, evoking deep-seated changes of metabolism. In the ontogeny of plants it is possible to make out phases of extra sensitivity to irradiation. Limited doses show a stimulating action on growth and development of agricultural crops. Large doses produce definite harm. The additive nature of the effect of irradiation and soil quality was emphasized.

On questions of technique there were a number of speeches accompanied by demonstrations of apparatus and films.

I. N. Birukov (Department of Scientific Cinematography, Moscow State University) gave a detailed description of a new application of his own technique of vital fluorescence and cinemicrography for the study of radiation injury in uni- and multicellular organisms. The results obtained commend the determination of the changes of brightness of the luminescence of fluorochromed objects as a new radiological technique. Birukov demonstrated portions of two-colour films to illustrate his lecture.

I. N. Verkhovskaia dwelt on the requirements demanded by work with radioactive substances, the construction and equipment of radiochemical laboratories, disposal of radioactive wastes, etc.

The question of dosage of ionizing radiations occupies an essential place in radiobiological investigations. Present-day ideas on doses of penetrating radiations and methods of determining them formed the subject of a paper by V. G. Khonazuk (Department of Biophysics, Moscow State University). Under his direction a demonstration of dosimetric apparatus of Soviet origin was given in the Department of Biophysics during the period of the Conference.

I. A. Terskov and I. I. Gitel'son (Krasnoiarsk) presented their method of determining the erythrogram—the distribution of erythrocytes by stability to external haemolytic agents. The method has prospects for following and defining the course of acute radiation injury.

In discussion the participants in the Conference debated the communications they had heard. In connexion with the speech of Benevolenskii, N. I. Zolotova (Moscow State University) and A. I. Polivoda (Institute of Biophysics, Academy of Medical Sciences of the U.S.S.R.) put forward the results of electron-microscopical investigations of liver homogenates.

Kalmanson (Botkin Hospital) called attention of members to the possibility of applying to radiobiological investigations the method of paramagnetic electronic resonance for the study of free radicals. Kalantarov (Moscow State University) touched on the question of the radiosensitivity of the nervous system and Prof. Livanov (Academy of Medical Sciences of the U.S.S.R.) gave the results of his

experiments on irradiation of the heels of rabbits, in relation to the existence of the phenomenon of impulseless signalization. In the discussions, questions of the interpretation of radiobiological experiments, the position of the target theory in present-day radiation biophysics, and also some aspects of the history of mathematical radiobiology (Prof. S. N. Ardashnikov and V. I. Korogodin) were touched on.

An interesting discussion on Dubinin's paper arose between Prof. S. I. Isaev (Department of Genetics, Moscow State University) and V. V. Khvostova (Institute of Biophysics, Academy of Sciences of the U.S.S.R.). Isaev expressed disagreement with the basic principles assumed in Dubinin's thesis. Admitting the existence of radiogenetic mutations, Isaev warned the Conference against the misuse of radiation methods in selection and seed raising. Quoting the works of foreign geneticists (Gustavson, Muller and others), Isaev expressed the view that irradiation of plants by ionizing radiations evokes instability of heredity rather than stimulation of growth and development, or the appearance of agriculturally useful characters. Khvostova recalled the latest works of Gustavson, Alikhanian and other investigators on the utilization in practice of useful radiomutants, and of the effect of stimulation. Genetics and selection form two independent fields of science—two steps in obtaining new forms. Radiation is one of the factors acting on hereditary structures, allowing the geneticist to establish new mutants while the selector has the task of adapting these forms to the needs of agricultural practice by use of selection, crossing and a number of other methods.

In the concluding address B. N. Tarusov remarked on the increase of interest in the present problem of the biological action of radiations on the part of workers in higher educational institutions. Tarusov asked the members of the Conference to associate themselves with the working out of the biophysical and biochemical mechanism of the action of ionizing radiations.

V. I. KOROGODIN
G. G. POLIKARPOV

Translated by T. R. PARSONS

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DEVELOPMENT OF BIOPHYSICS IN THE FORTY YEARS FOLLOWING THE GREAT OCTOBER SOCIALIST REVOLUTION*

A. M. KUZIN and G. M. FRANK

SOON after the establishment of Soviet rule, against the background of a general rapid development of science in Soviet Russia, new regions of knowledge began to be built up as a result of the contact of various disciplines, such as physical chemistry, biological chemistry and biological physics. The contact between physics and biology took place on two levels. On the one hand, research was carried out on the physical properties of and the physical processes in living systems, and on the other, the action of physical agents on these systems was studied.

Among the physical factors, the action of ionizing radiations on the organism was especially intensively studied because of its exceptional importance in medical practice. Because of the subsequent progress of atomic physics and the mastering of atomic energy this factor became of prime importance and led to the separation of an independent discipline which received the name "radiobiology" and which is very closely connected with biophysics.

During the first years of Soviet rule, foresight into the future trends of science and a clear understanding of the advantages of using physics in biology and medicine, not only led to the formation of new border disciplines on the basis of those already existing, but also stimulated the organized moulding of institutes of a completely new type. Here, in the first place, one should name the Institute of Biophysics of the Ministry of Public Health of the U.S.S.R., which arose on the basis of the prerevolutionary Institute of Physics and which, according to the conception of its founder, Academician P. P. Lazarev, directed the efforts of the trained physicists collected there to the solution of the principal problems of biology.

Of great importance for the development of radiobiology, apart from its value in connexion with practical problems such as X-ray diagnosis, and X-ray therapy and radiotherapy, was the founding of another centre, the Institute of Röntgenology and Radiology in Leningrad, which was uninterruptedly directed by Prof. M. I. Nemenov for about 30 years.

The school of Academician Lazarev preserved its leading role in the field of biophysics up to the death of its founder at the beginning of World War II.

In justice we should consider Lazarev to be the creator of Soviet biophysics since he has not only done most work in the field of biophysics, but has has also formulated the general tasks of this young science and defined its subject and its special methodological procedures. His ionic theory of excitation, which plays an important role in understanding the biophysics of living processes, at a definite stage of development of science, was fundamental for the development of the whole trend in the field of research on the mechanisms of the phenomenon of excitation. At the same time it

* Biofizika **2**: 5, 545–551, 1957 [Reprint Order No. BIO 72].

served as a starting point for the study of the problem of the biophysics of vision, which was especially widely developed in Lazarev's school.

Certain physiological investigations should be considered as directly bordering on biophysics, particularly those carried out by Academician L. A. Orbeli and his students on the functions of sense organs. In this connexion one should recall the work of one of the pioneers of Soviet electrophysiology, Prof. A. A. Samoilov, who studied electrophysiological processes of nerve excitation.

The work of a number of the most outstanding Soviet physicists, who began their activity in the Institute of Biophysics under the direction of Lazarev, was very important for the subsequent development of biophysics. The experiments of Academician S. I. Vavilov acquired world-wide fame; using the eye as a light-sensitive apparatus he showed, directly, that it is possible visually to observe the discrete nature of light. Investigations in the field of vision were further pursued; among these one should mention, in the first place, the work of S. V. Kravkov on the interaction of the sense organs and the work of N. T. Fedorov on the mechanism of colour vision.

S. N. Rzhevkin and L. A. Andreev developed the bases of the biophysics of the auditory apparatus. The investigations of A. G. Gurvich, who discovered mitogenetic rays in 1923 and who showed that they were ultra-violet rays, evoked lively discussion in the world literature.

At the beginning of the twenties, G. A. Nadson and his co-workers, when studying the action of radiations on micro-organisms and plant cells, made a discovery of enormous importance: they established that it was possible to obtain hereditary changes by irradiating the parent organism. Two years later, Muller published the results of experiments which showed that, by X-ray irradiation, hereditary changes could be evoked in fruit flies, and he was awarded the Nobel prize for this work.

At the same time, in the Institute of Röntgenology and Radiology, investigations were started which formed the basis of the original Soviet trend in radiobiology. In the work of P. S. Kupalov, and also of Nemenov, it was shown (and subsequently developed) that the nervous system has a high functional sensitivity to radiation. This opposed the ideas of the majority of foreign investigators on its considerable resistance, ideas based only on comparative morphological analysis of various organs and tissues.

In these same years, side by side with Moscow and Leningrad, investigations in the field of radiobiology were started in many other towns of the Soviet Union. Here we should primarily mention the work of N. M. Voskresenskii in the Experimental Biology Laboratory of the Kiev X-ray Institute and the work of A. V. Rentev and I. P. Mishchenko in the Ukraine Röntgenology and Radiology Institute in Kharkov.

To 1936 pertains the organization, in the All-Union Institute of Experimental Medicine (V.I.E.M.), of a department of biophysics and photobiology (G. M. Frank) in which, on the one hand, the action of physical factors on the organism and, on the other, the physical and physico-chemical processes lying at the basis of vital phenomena were studied. The most important aspect of the researches was the development of a new approach to the specificity of the biological action of ultra-violet rays of different wavelengths. The disclosed features of this specificity were explained by analysis of the photochemical basis of the action of the ultra-violet rays. In the same

Institute, by the method of X-ray structure analysis, the first work on the ultrastructure of living tissues was carried out and the basis of the application of isotopic indicators was laid.

The investigation of those physico-chemical processes which are fundamental in vital phenomena attracted the attention of many investigators in those years. Problems of permeability, the role of surface phenomena in biological processes, colloid–osmotic phenomena, sorption processes in living systems, were worked on by D. N. Nasonov, V. Aleksandrov, P. A. Rebinder, B. V. Deryagin, D. L. Rubinshtein and others.

In the investigations of Rubinshtein and his co-workers the theory of the biological action of ions was developed and clear quantitative criteria were proposed of the phenomena of sensitization, synergism and antagonism of ions in salt mixtures. The theory of "one-sided" permeability of tissue membranes was put forward on the basis of the principle of stationary states in biological systems.

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To this same time (1928–1939) should be referred a series of beautiful studies on the biomechanics of motion of marine animals carried out by V. V. Shuleikin and his co-workers and also the work of N. A. Bernshtein on human biomechanics.

In 1935 the Agrophysical Institute (A. F. Ioffe) was organized in the system of the V. I. Lenin All-Union Academy of Agricultural Sciences in order to make systematic use of the achievements of physics in agrobiology. This considerably helped the development of light-culture, the more rational use of fertilizers and the solution of other problems which faced agriculture.

Thus, in the pre-war period contacts between physics and biology were established, a tradition of biophysical and radiobiological experiment was founded, and a nucleus of research workers, using physics in one or other way for the solution of biological problems, was developed.

After the end of the war the advances in nuclear physics, which led to the discovery of the method of using atomic energy, the construction and use by the U.S.A. of an atomic weapon, the building in the U.S.S.R. of power stations using atomic fuel, the construction of powerful accelerators and the wide introduction of radioactive isotopes into scientific and technical investigations, confronted biophysics with a series of new problems and aided the increased development and separation as an independent discipline of one of the branches of biophysics—radiobiology.

Radiobiology became the theoretical basis for the development of methods of protection against radiation, for the prophylaxis and treatment of radiation sickness, and also for the practical utilization of atomic energy in medicine and agriculture.

The Radiation Laboratory, reorganized into the Institute of Biophysics of the Academy of Medical Sciences of the U.S.S.R., was founded in the Academy of Medical Sciences of the U.S.S.R. (G. M. Frank, subsequently A. V. Lebedinskii). A laboratory for isotopes and radiations was founded in the Division of Biological Sciences of the Academy of Sciences of the U.S.S.R. (A. M. Kuzin). A biophysical laboratory was organized in the K. A. Timiryazev Agricultural Academy (V. M. Klechkovskii). In the Ukrainian Academy of Sciences a biophysical laboratory (A. A. Goradetskii) began working at the Bogomolets Institute. Radiobiological work was expanded at the Central Scientific Research Röntgen-radiology Institute of the Ministry of Public Health of the U.S.S.R. (M. N. Pobedinskii).

At the A. N. Bakh Institute of Biochemistry of the Academy of Sciences of the U.S.S.R. a biophysical laboratory was created for the study of photosynthesis (A. N. Terenin). An electron microscopy laboratory (A. E. Kriss) has been started in the Division of Biological Sciences of the Academy of Sciences of the U.S.S.R.

In 1952, on the basis of the Laboratory for Isotopes and Radiations of the Division of Biological Sciences of the Academy of Sciences of the U.S.S.R., the Biophysics Laboratory of the I. P. Pavlov Institute of Physiology of the Academy of Sciences of the U.S.S.R. and S. V. Kravkov's Laboratory for Vision and the Laboratory for Speech and Hearing, there was created the Institute of Biophysics of the Academy of Sciences of the U.S.S.R. (A. M. Kuzin). In order to train numbers of biophysicists, a chair of biophysics (B. N. Tarusov) was founded at the Moscow State University in 1953.

Biophysical investigations are carried out in the State Optical Institute (E. M. Brumberg, G. N. Rautyan), in the physics department of the Military Medicine Academy (N. T. Fedorov), in the department of animal physiology of Moscow State University (Kh. S. Koshtoyants), in the Biophysical Laboratory of the Ural branch of the Academy of Sciences of the U.S.S.R. (N. V. Timofeev-Resovskii), in the physics department of the V. I. Lenin Moscow State Pedagogical Institute (N. N. Malov), in the P. F. Lesgaft Natural Science Institute (M. A. Khenokh), in the I. P. Pavlov Institute of Physiology of the Academy of Sciences of the U.S.S.R. (G. V. Gershuni), in the Gorky State University (V. A. Opritov) and in many other scientific and educational establishments.

For the development of radiobiological research, radiobiological laboratories have been created in the Academy of Sciences of the U.S.S.R.: in the Institute of Microbiology (M. N. Meisel'), in the A. N. Bakh Institute of Biochemistry (A. G. Pasynskii), in the Institute of Genetics (A. N. Nuzhdin), in the A. N. Severtsov Institute of Animal Morphology (E. Ya. Graevskii), in the I. P. Pavlov Institute of Physiology (I. A. Chetvernikov), in the Institute of Higher Nervous Activity (I. A. Plantkovskii), Also, radiobiological work is carried out in the universities, institutes of the academies of sciences of the union republics and in a large number of medical science establishments.

The founding, two years ago, of a special organ of the Academy of Sciences of the U.S.S.R.—the journal "Biofizika"—has undoubtedly been of great importance for the development of biophysics and radiobiology and for the consolidation of investigations in these fields.

Corresponding to the general advances of Soviet biological science, which has attained pre-eminence in the development of the idea of "nervism", considerable advances in the biophysical field have recently been made on problems of excitability, irritability and functions of the sense organs.

In the numerous papers of D. A. Nasonov and his co-workers a new trend of research in the field of cell physiology has crystallized. It has been established by various methods that the basis of the reaction of living protoplasm to external agents is a special change in the physico-chemical state of the protein complexes—a reversible denaturation. This has enabled Nasonov to reach a conclusion about the physico-chemical basis of the reaction of a cell to stimulating and injuring effects.

For the first time, by the work of Frank and his co-workers, the changes were detected which arise on excitation of nerve conductors and which are propagated

along the nerve fibre at the speed of conduction of the excitation. This, side by side with the electrophysiological method, disclosed the possibility of analysing excitation phenomena by studying optical and visco-elastic properties and has definitely changed our concepts of their nature. To the same field of research belongs the application of structural analysis to "dynamic molecular morphology", using X-ray diffraction and electron microscopy studies of tissues whose structures have been "arrested" in various functional states. The results obtained have enabled general opinions to be expressed about the role of the mobility of biological structures.

Out of the work carried on in the department of medical physics of the S. M. Kirov Military Medicine Academy (N. T. Fedorov), it is necessary to name first of all a long series of investigations in the field of colour contrast. In these investigations the dependence of the magnitude of the simultaneous colour contrast on the magnitude of the inducing field and on its brightness and saturation, was quantitatively studied. It was shown, both for simultaneous and successive colour contrast, that contrasting colours regularly differ from complementary, coinciding with them only at four "special" points of the spectrum and at two points in the purple region. By studying the effect of passing direct current through the eye-ball on simultaneous colour contrast, they succeeded in showing the "perielectrotonic" character of these phenomena.

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In the laboratory of vision of the Institute of Biophysics of the Academy of Sciences of the U.S.S.R. (D. N. Nyuberg) new, and very important, facts have been obtained on the probability of the existence in every cone of the human eye of all three receptors of colour vision. Hence, the question arose of the possibility of transmission of three different types of signal along one and the same fibre. This possibility was confirmed by direct experiment in a study of the electrical signals on a single fibre of the optic nerve of the frog. The great novelty of these facts opens up a new trend in the study of the material basis of visual perception.

A new field of application of physics to biology—the investigation of photosynthesis (A. N. Terenin, A. A. Krasnovskii)—is being developed in the A. N. Bakh Institute of Biochemistry. The principles established by Academician A. N. Terenin in his long-continued investigations devoted to fluorescence have proved fruitful for the discussion of the conversion of energy absorbed by green leaves, the mechanism of photochemical reactions and the participation of energy migration in the process.

The investigations carried out by L. A. Blyumenfel'd on the spectra of the electron paramagnetic resonance of proteins are very important for understanding the physical and physico-chemical processes taking place in the living substrate. The anomalously narrow absorption band detected by him is characteristic of protein systems in a state of catalytic activity and disappears in inactivated enzymes. This phenomenon can be interpreted as evidence for the existence in protein systems of free-electron conductivity. Hence, comparisons with the available facts suggest that energy migration is some general property of living structures and protein systems in an elevated state of chemical activity.

Important results on the uniqueness of the structure of the living, physico-chemical state of a heterogeneous substrate and on the localization of enzyme systems in the structures have been obtained by the method of the action of ultrasonics on biological objects (I. E. El'piner).

Facts have emerged which indicate that properties, recalling the features of semiconducting systems and including processes of energy migration, are in some measure also characteristic for the substrate of the elements of nervous tissue, in particular the visual receptors. This brings together the different trends of biophysics, of which we spoke earlier, creating a completely new and original system of concepts.

It is very characteristic of the modern stage of biophysics that wider use is constantly made of various modern physical methods, opening up new perspectives in the study of the properties of and the processes in the living system.

Of especially great importance is the development and utilization of methods of investigating submicroscopic structures, particularly by electron microscopy of ultrathin sections and also by X-ray structure analysis of biological material and organic substances. Great success has been achieved in the field of ultra-violet microscopy, which has furthered the development of ways of founding a sort of "topographical biochemistry", and also in other methods of physical investigation of chemical structure or chemical processes. One could mention a whole series of such methods, starting from molecular spectroscopy and ending with the method of paramagnetic electron resonance.

The use of isotopic indicators occupies a special place in these methodological procedures; at first they were applied in specialized laboratories and then gradually introduced into biological establishments of the most varied types. Both the quoted and other methodological procedures have essentially re-armed modern biology, enabling it to penetrate more deeply into the intimate processes of the material foundations of vital phenomena.

Leaving aside applied and purely medical problems of radiobiology, it should be noted that the pathological physiology and pathological morphology of radiation sickness has been studied on a great deal of experimental material in recent years, and theories of the pathogenesis of radiation sickness have been developed (L. A. Orbeli, P. D. Gorizontov, A. N. Kraevskii, G. M. Frank and others).

When studying radiation effects on higher animals much attention has been paid to the action of ionizing radiation on the nervous system (L. A. Orbeli, M. N. Livanov, F. P. Maiorov, P. I. Lomonosov, N. M. Livshits, P. F. Minaev and others). The facts on the high radio-sensitivity of the nervous system have been experimentally confirmed and broadened and valuable material has been obtained on the regularities of the radiation effects on the nervous system due to various types of agent. In this connexion it should be especially noted that, by means of delicate electrophysiological research methods (M. N. Livanov), the characteristics of the reactions of the various links of the central nervous system to the effects of ionizing radiations have been demonstrated. Investigations on the reactions of the vascular system are of great importance for the understanding of the mechanism of radiation sickness (G. M. Frank, A. V. Lebedinskii). The effect of ionizing radiations on the development of immunity and on the resistance of an organism to infections has been studied (V. L. Troitskii).

One of the central problems of modern radiobiology which is agitating mankind is the effect of irradiation on posterity. Valuable research on this problem has been carried out by N. I. Shapiro and N. I. Nuzhdin. In order to construct a theory of the biological effect of irradiation it is especially important to study the effect of

radiation on elementary living processes. From this point of view investigations on the effect of radiations on the cell, carried out by the application of new methods of luminescence microscopy (M. N. Meisel'), are very interesting, as are also delicate investigations on the first-stage and primary processes of the radiation reaction of an organism (B. N. Tarusov, A. G. Pasynskii, A. M. Kuzin, M. A. Khenokh, Ya. L. Shekhtman, L. Kh. Eidus and others).

The problems of the primary processes have been developed in many directions. The presence of chain reactions in the lipid phase of organs and tissues and the appearance of peculiar, toxic, high-polymer substances, have been detected and investigated in detail (B. N. Tarusov). A method of sorption of radioactive amino acids (A. G. Pasynskii) has been used to observe initial denaturation processes in irradiated protein solutions. A study of the change in the infra-red and ultra-violet spectra of irradiated proteins showed the prevalence of aggregation processes due to the effect of radiation. The possibility of sensitizing nucleic acids to the action of ionizing radiation, the parallelism between radio-sensitivity of a tissue and the degree of depolymerization of DNA after exposure, and the role of a change in the native condition of high-polymer substances in the radiation reaction of an organism, have all been shown (A. M. Kuzin). A change in the isoelectric point of nuclear and mitochrondrial proteins has been demonstrated (A. L. Shabadash). The role of "complex-formation" in the protective effect of substances of high molecular weight has been shown (L. Kh. Eidus). Early changes in the biocolloids in cells and tissues were shown by the method of luminescence microscopy (M. N. Meisel', V. A. Sondak), which finds wide application.

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Electrophysiological investigations of the effect of radiation on the nervous system have shown that there is practically no latent period for this reaction and that functional changes of the nervous system are observed in the very first seconds after the beginning of exposure (M. N. Livanov, N. A. Aladzhalova, Yu. G. Grigor'ev). The development and application of a method of *in vivo* tissue polarography (A. D. Snezhko) has disclosed the disturbance of tissue oxidation processes which sets in, primarily, in the part of the tissue which is directly exposed to the effect.

All this makes penetration possible into the so-called "open" period of the radiation reaction of animals, deciphering processes which are developing at this stage.

The study of the radiobiology of plant organisms has been of great importance for practice and theory: the general laws of the effect of radiation on growth and development of plants have been worked out (V. M. Klyachkovskii), the fundamentals of the effect of radiation on the metabolism of plants have been studied (N. M. Sisakyan), the activating effect (under known conditions) of radiation on higher plants has been detected (L. P. Breslavets, N. V. Timofeev-Resovskii, Zhezhel'). Here we should also refer to a whole series of cyto-biochemical studies on the action of radiation on micro-organisms, radio-sensitive structures in the microbe cell, and disturbances of energetic processes have been demonstrated (M. N. Meisel').

The wide development of radiobiology in the U.S.S.R. has enabled the leading radiobiological laboratories to generalize the results obtained and to demonstrate the development of original Soviet trends at the session of the Academy of Sciences of the U.S.S.R. on the peaceful uses of atomic energy (1955), at the International

Conference on the Peaceful Uses of Atomic Energy in Geneva (1955). At the Scientific–Technical Conference on the Application of Isotopes and Radiations in the National Economy and Science (1957) it was possible both to sum up and theoretically generalize current investigations (A. V. Lebedinskii, M. N. Meisel', A. M. Kuzin, G. M. Frank, P. F. Gorizontov).

The basic task of biophysics is the close co-operation of physicists and chemists with biologists so as to uncover the hidden secrets of life. In the 40 years since the Great October Revolution great and fruitful work has been done along these lines. However, all the growing demands which are made on biophysics by medicine, agriculture and such vigorously expanding branches of our national economy as atomic industry, compel all workers in this field to be still more exacting in their work and to strive to give everything that we have a right to expect from this promising young discipline.

Translated by J. DAINTY

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ELECTRON PARAMAGNETIC RESONANCE SPECTRA OF BIOLOGICAL OBJECTS—I*

ACTION OF IONIZING RADIATION ON DRY AMINO ACIDS, PEPTIDES, PROTEINS AND LYOPHILIZED TISSUES

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(*Received* 6 *May* 1957)

ZAVOISKII's (1945) discovery of electron paramagnetic resonance (e.p.r.) [1] presented chemists and biologists with a radically new research tool. As e.p.r. spectroscopy is as yet unfamiliar to many biophysicists and biochemists, this paper is devoted to presenting the fundamentals and possibilities of the method. The original experimental data presented here relate to spectra of free radicals formed by the action of ionizing radiation on dry amino acids, peptides, proteins and lyophilized tissues. Our e.p.r. spectra of native proteins and enzyme systems have been published in part by one of us [2] and will be fully published.

THE ESSENCE OF E.P.R.

E.P.R. spectra result from transitions induced by a high-frequency electromagnetic field between electronic levels subjected to Zeeman splitting in a magnetic field. E.P.R. spectra can only be produced if the particles in the object show net electron magnetic moments. The essence of the phenomenon can be demonstrated in the very simplest case of a free electron. As a rule the e.p.r. spectra of the free radicals of interest to us approximate to this ideal case.

* Biofizika **2**: 5, 552–565, 1957 [Reprint Order No. BIO 73].

An electron has an inherent spin angular momentum of $s=\sqrt{[\frac{1}{2}(\frac{1}{2}+1)]}\,h/2\pi = h\sqrt{3}/4\pi$ g cm^{2}sec^{-1}, $h=6{\cdot}62\times10^{-27}$ erg sec (Planck's constant). If there is a preferred direction in space z (e.g. the molecular axis or the direction of an external magnetic or electric field, etc.) the projection of the spin s^z on this direction can only take the values $\pm h/4\pi$ (or $\pm\frac{1}{2}$ in units of $h/2\pi$). This spatial quantization means that the spin can only be orientated in certain definite ways in space. The maximum value of s^z is termed the spin quantum number, usually denoted by s. For free electrons $s=\frac{1}{2}$. For a system containing several electrons with parallel spins the s values of the individual electrons combine to give a resultant S. Thus for the O_2 molecule, with two unpaired electrons, $S=1$. The total spin S^* is $\sqrt{S(S+1)}\,h/2\pi$; its projection on any fixed direction in space can take $2S+1$ values: S, $S-1$, $S-2$, ... (in units of $h/2\pi$).

A magnetic moment μ is associated with the spin S^* and μ/S^* is denoted by g, i.e.

$$\mu = g\,S^* = g\sqrt{S(S+1)}\,h/2\pi. \tag{1}$$

For free electrons $g=e/mc$, e and m being the charge and mass of the electron and c the velocity of light. The quantity g is usually expressed in units of $e/2mc$. In these units $g=2$ for a free electron. The projection of μ on z, μ^z, is quantized and can take the value $(e/mc)S^z$. For free electrons $\mu^z=eh/4\pi mc$, and $eh/4\pi mc$ the Bohr magneton (β) is $0{\cdot}9237\times10^{-20}$ ergs/G. In general the total momentum vector $\mathcal{J}$ for the system (the vector sum of the orbital and spin vectors) corresponds to a magnetic moment $\mu=g\sqrt{\mathcal{J}(\mathcal{J}+1)}\,\beta$, $\mathcal{J}$ being the internal quantum number specifying the moment and g is the Landé factor, given by

$$g = 1 + \frac{\mathcal{J}(\mathcal{J}+1)+S(S+1)-L(L+1)}{2\mathcal{J}(\mathcal{J}+1)} \tag{2}$$

for Russel–Saunders coupling. Here S is the resultant spin quantum number and L the resultant orbital quantum number. For a free electron $L=0$, $S=\frac{1}{2}$, $\mathcal{J}=\frac{1}{2}$, so $g=2$, and g is also called the gyromagnetic ratio for a free electron.* As stated above the projection of μ on any fixed direction is quantized and can have $2\mathcal{J}+1$ values, corresponding to magnetic quantum numbers $M_{\mathcal{J}}=\mathcal{J}, \mathcal{J}-1, \ldots -\mathcal{J}$. At zero external field (electric or magnetic) states of differing μ^z have equal energies, i.e. are degenerate. This degeneracy is eliminated by a time-invariant magnetic field. Roughly speaking, the elementary magnets tend to lie along the field direction rather than in the opposite direction. Hence energy levels related to orientation of the moment appear in a steady magnetic field. For a free electron system this can be represented schematically as below:

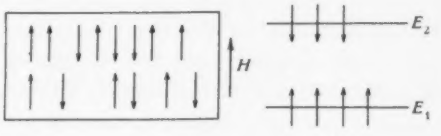

FIG. 1.

* The exact value of g for a free electron is 2·0023.

If $\mathcal{J}>\frac{1}{2}$ several energy levels appear. Spontaneous transitions between these levels scarcely occur. But if an alternating magnetic field is also present (i.e. if the specimen is placed in a high-frequency electromagnetic field) interlevel transitions will be produced. The selection rule

$$\Delta M_{\mathcal{J}} = +1 \tag{3}$$

shows that transitions can only occur between adjacent levels. For a transition to occur, the difference in energy ΔE between two adjacent levels and the energy of the electromagnetic quantum $h\nu$ must be equal. For a level of magnetic quantum number $M_{\mathcal{J}}$ the energy due to μ interacting with a steady magnetic field of strength H is $E=gM_{\mathcal{J}}(h/2\pi)H$. Here $gM_{\mathcal{J}}(h/2\pi)$ is the projection of μ on H in cgs (magnetic) units. Then $\Delta E=gM_J(h/2\pi)H-g(M_{\mathcal{J}}-1)(h/2\pi)H=g(h/2\pi)H$. If, as is always done, g is expressed in units of $e/2mc$ then $\Delta E=g(e/2mc)(h/2\pi)H$. Thus transitions induced between magnetic energy levels by the electromagnetic field will occur if

$$\nu = g\beta H/h. \tag{4}$$

The probabilities of transitions involving either absorption or emission of energy are equal:

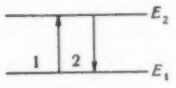

FIG. 2.

(transition types 1 and 2).

Under normal conditions, the distribution between E_1 and E_2 is of the Boltzmann type, the number of electrons in the lower state E_1 (spin along the field) being somewhat greater than in E_2 (spin against the field). Hence $E_1 \rightarrow E_2$ transitions are more frequent than $E_2 \rightarrow E_1$ and electromagnetic energy is absorbed. Magnetic resonance absorption* occurs. Equation (4) shows that absorption can be effected in two ways: (1) by varying ν at fixed H; and (2) by varying H at fixed ν (monochromatic radiation). The second method is commonly used in practice. The position of the absorption line is dependent on g. For free electrons ($g=2$) the absorption lies at $\nu\approx 9\times 10^9$; c/s at $H=3300$ G, this being in the microwave range ($\lambda\approx 3{\cdot}3$ cm). Thus, e.p.r. spectra can be studied in the centimetre wave region at fields of a few thousand oersted. The e.p.r. lines vary in position, width and shape. These characteristics will be considered individually.

1. *Position.* The position of a line, or more precisely its g, is one of its most important parameters. In h.f. fields, the g may differ from the Landé factor. Hence this factor is normally termed the spectroscopic splitting or simply the g-factor.

If the electron moment is of purely spin origin, or if the orbital moment is completely suppressed, the g-factor should be that for free electrons, i.e. 2·0023. Thus, any shift from $g=2{\cdot}0023$ must be due to spin-orbit coupling. The ground states of organic free radicals are usually doublets, but any further shift can only be due to excited states. The shift increases with the strength of the spin-orbit coupling and

* In describing these magnetic absorption phenomena we commenced from purely quantum-theory concepts. From the classical viewpoint the magnetic moment vector precesses round H (Larmor precession). Absorption occurs if the alternating field resonates with the precession (frequencies equal). Hence the term "resonance absorption".

with the depth at which the excited state lies. As a rule the Δg in free radicals containing no atoms heavier than C, N, O and H do not exceed 0·004. For radicals containing *S*, $\Delta g \approx 0{\cdot}02$ usually. For metal ions *g* may differ much from 2. Thus, *g* for the e.p.r. lines of iron in haemin and methaemoglobin is close to 6 [3]. Interesting conclusions have been drawn concerning the natures of the bonds to the iron in these compounds from the value of *g*.

2. *Width.* Broadening in e.p.r. lines is mainly determined by two factors, spin-lattice and spin-spin interactions. Spin-lattice interactions, i.e. interactions between the spin magnetic moments and the surrounding electric fields (of the lattice in a solid, of the groups in molecules, etc.) determine the lifetime of the excited magnetic state, or in other words the time required for spin-reversal and return to the ground state. The stronger the interaction the shorter the relaxation time. Heisenberg's uncertainty principle gives $\Delta E \cdot \Delta t > h/2\pi$, so the less Δt the greater ΔE, i.e. the greater the e.p.r. line-width. Spin-lattice interaction is of course reduced at low temperatures. Very extensive spin-lattice interaction broadening may occur in compounds with strong spin-orbit coupling. In UF_6 the e.p.r. line-width is about 5000 G. For most of the compounds of interest here the spin-lattice interaction is not the limiting factor in the line-width.

Each unpaired electron experiences the somewhat non-uniform magnetic field of all the other unpaired electrons in the specimen, as well as the external field. The effective fields at the various electrons then differ and so the e.p.r. line is broadened. This spin-spin broadening is susceptible of fairly strict quantum-mechanical calculation [4, 5] and very frequently determines the e.p.r. line-width.

Van Vleck [5] has shown that exchange interactions between unpaired electrons narrow the e.p.r. lines. This exchange interaction in solid stable aromatic free radicals causes the extremely narrow lines observed. Thus, the half-width $\Delta H_{\frac{1}{2}}$ for diphenylpicrylhydrazyl is less than 2 G.

Anderson [6] has shown theoretically, and Walter *et al.* [7] experimentally, that rapid translation (i.e. electron delocalization involving many centres) can also produce line narrowing. As we shall see later, this is of great importance in interpreting e.p.r. spectra for biological objects.

3. *Shape.* We will not pause over deviations from line symmetry, or fine structures due to loss of degeneracy caused by the electric fields of the crystal, or anisotropy in *g*-factors. These topics have been dealt with adequately in the literature (see, for instance, [8]). We pass directly to the topic of most interest in chemistry and biology —hyperfine structures in e.p.r. spectra.

Many nuclides have a nuclear spin and hence an inherent magnetic moment. These nuclear moments are some 10^3 times less than electronic ones. Interaction between the nuclear and unpaired electron spin moments causes line-splitting and hyperfine structures. The spins and moments (in nuclear magnetons) of certain nuclei are given below:

Nucleus	^{1}H	^{2}H	^{12}C	^{14}N	^{16}O	^{31}P	^{32}S
Spin	$\frac{1}{2}$	1	0	1	0	$\frac{1}{2}$	0
Moment	2·79	0·86	0	0·40	0	1·13	0

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Hyperfine structure formation will be considered in relation to the most important instance, proton-unpaired electron interaction. The spatial quantization rules show that the proton spin can have two orientations relative to the electron spin direction, giving projections on this direction (M_I) of $+\frac{1}{2}$ and $-\frac{1}{2}$. This either increases or reduces the total magnetic moment of the electron + proton system relative to the free-electron value. The e.p.r. line thus splits into two components lying somewhat above and below the field values for the zero-interaction case. The splitting is determined by the interaction energy. Thus, when an unpaired electron interacts with one proton, a doublet of equal-intensity components is produced. A schematic representation of the origin of the fine-structure is as follows:

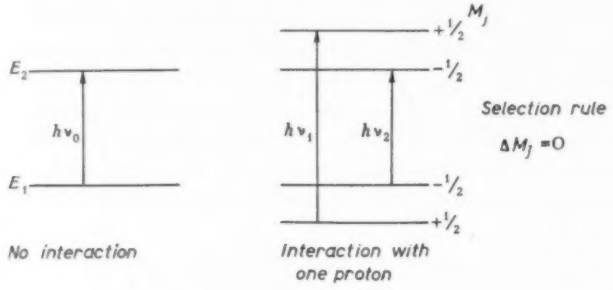
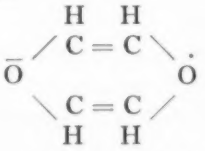


FIG. 3.

It is readily shown that, in general, when the unpaired electron interacts with n structurally equivalent protons we get a hyperfine structure consisting of $n+1$ equally spaced components with intensities in the ratios $1:n:n(n-1)/2 \ldots C_n^m \ldots n:1$. Thus, the semiquinone ion-radical



formed as an intermediate in the oxidation of alkaline hydroquinone solutions by atmospheric oxygen, has four equivalent protons and gives an e.p.r. spectrum consisting of five equispaced hyperfine components of intensities 1:4:6:4:1. Fig. 4 (1) shows a photograph of this spectrum presented on an oscilloscope. The very strong exchange interactions cause the lines to be very narrow (total splitting only a few gauss).

When structurally nonequivalent protons are present the spectra become more complex but can frequently be deciphered.

The considerable possibilities of using e.p.r. for solving detailed structural problems as well as for quantitative and qualitative studies on free radicals are thus evident.

PUBLISHED E.P.R. SPECTRAL DATA FOR BIOLOGICAL OBJECTS

Relative to the enormous number of physical and chemical studies performed with e.p.r. in recent years, almost nothing has appeared on biological objects. But

the few published data show the unforeseen possibilities revealed by using e.p.r. for solving biochemical and biophysical problems.

Blois [9, 10] studied the free radicals produced as intermediates in redox processes on certain biologically important quinone-type compounds (adrenalin, vitamin K_1, riboflavin, etc.). We have already noted the e.p.r. spectra of iron in haemin, haemoglobin and some of their derivatives [3]. The results indicate that the bonds to the pyrrol nitrogen are not ionic as was earlier supposed.

Commoner *et al.* [11] observed free radicals in lyophilized tissues and animal and plant fluids: this is of great interest. Fractionation experiments led them to associate the radicals with the proteins. Their concentrations were 10^{-6}—10^{-8} per g dry material. Functioning enzyme processes and illuminated green leaves showed much greater concentrations, destroyed by denaturation.

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One of us [2] has published data on the extreme narrowness of the lines from lyophilized tissues which indicates extensive electron delocalization in the protein molecule. The origins of the free electrons in proteins and their relation to energy migration were also considered.

Commoner *et al.* [12] have also given data on the effect on the e.p.r. spectrum of illuminating a chloroplast suspension. The rates of rise and fall of concentration on illumination were studied. They supposed that they were dealing either with intermediate radical states in substances such as diphosphopyridine nucleotide, or with the triplet state of chlorophyll.

Gordy *et al.* [13] deal with the free radicals produced by X-rays in certain amino acids and proteins. Very interesting data on the structures of the free radicals were obtained, together with information on the effect of oxygen and on the role of disulphide bonds in stabilizing the primary radiation products from proteins. Unfortunately typical proteins (with enzymatic activities) were not studied, the authors restricting themselves to silk-type proteins from hair, horn, feathers, hide and fish-scales. Lines tens or hundreds of gauss wide were obtained.

In our work we studied systematically the e.p.r. spectra of the free radicals produced by γ-irradiating dry amino acids, peptides, proteins, lyophilized tissues and nucleic acids. Of the 25 preparations we used, 4 had earlier been studied by Gordy *et al.* [13].

EXPERIMENTAL METHODS

The e.p.r. spectrometer was built by us in Grashchenko's laboratory of the Biological Sciences Division, Academy of Sciences of the U.S.S.R.; it was of the normal reflection type with wide-range magnetic field modulation and wide-band amplification. The cavity resonator was cylindrical and excited in the H_{111} mode; it had a non-contacting quarter-wave piston.* The recordings were made by photographing the oscilloscope screen. The apparatus has been described in detail before [2]. The absolute sensitivity extended to 10^{-9}M (diphenylpicrylhydrazyl).

Most of the amino acid preparations were kindly provided by Professor Kaplanskii and the trypsin, trypsinogen and casein preparations by Professor Shlygin. The tissues were lyophilized some time after removal without strict temperature control, and none of them therefore gave e.p.r. spectra before irradiation.

* We are indebted to A. E. Nudel'man for assistance with the construction and for making the high-grade cavity resonators.

The irradiations were done with a cobalt beam, i.e. with γ-rays much harder than the radiation from the 50 kV X-ray tube Gordy *et al.* used. The preparations were all given the same dose, which was varied from 10^6 to 10^7r. Unless stated to the contrary, the irradiations were done *in vacuo*.

RESULTS

Amino acids

1. *Alanine** (Fig. 4 (2)). The spectrum is a symmetrical quintet with intensities 1: 4: 6: 4: 1, indicating that the free electron interacts with four equivalent protons: the *g*-factor of the central peak is 2·0069 ± 0·001. Splitting between adajcent components is 22 ± 3 G, overall splitting (ΔH_{1-5}) 88 G (Gordy gives 95).

2. *Phenylalanine* (Fig. 4 (3)). Single signal. Hyperfine structure not resolved. $\Delta H_{\frac{1}{2}} = 20 \pm 2$ G.

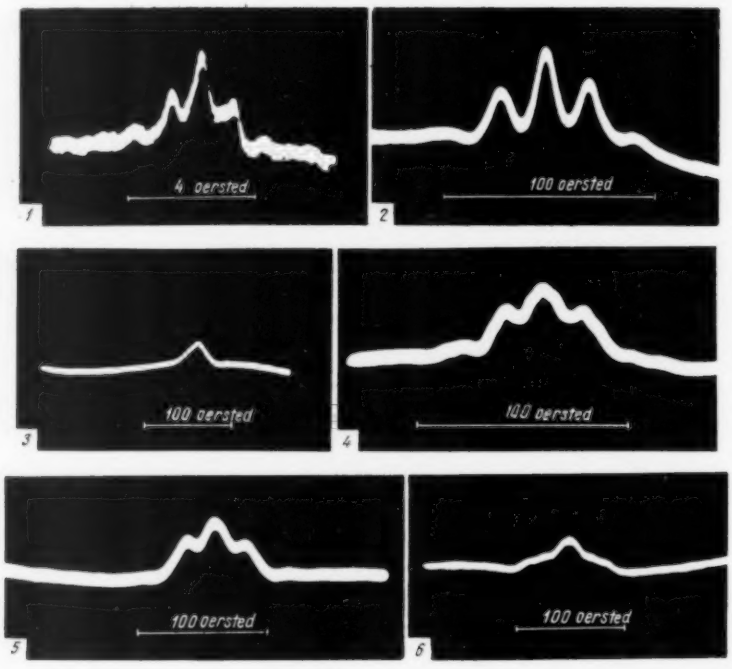


FIG. 4.

3. *Valine** (Fig. 4 (4)). Complex symmetrical signal in which 11 hyperfine structure components are visible, some being very poorly resolved. Components 2, 4, 6, 8 and 10 form a symmetrical quintet with intensities in the ratio 1: 4: 6: 4: 1 and with $\Delta H_{2-10} = 84 \pm 4$ G; $\Delta H_{1-11} = 103$ G, i.e. less than given by Gordy (160 G); the *g*-factor of central peak is 2·005 ± 0·002. We agree with Gordy's supposition that this is a quintet and septet superimposed with their central peaks coincident.

4. *Lysine* (Fig. 4 (5)). Symmetrical quintet. Splitting larger at the edges than the centre: $\Delta H_{1-2} = \Delta H_{1-5} = 33 \pm 3$ G; $\Delta H_{2-3} = \Delta H_{3-4} = 23 \pm 3$ G; $\Delta H_{1-5} \approx 112$ G. End

* Denotes preparations studied by Gordy *et al.* [13].

peaks less strong than the theoretical 1: 4: 6: 4: 1 relation would indicate; *g*-factor of central peak 2·004 ±0·001.

5. *Norleucine* (Fig. 4 (6)). Complex signal in which 5 components with the unusual intensity ratios 3: 4: 9: 4: 3 are seen, but the central peak may be compound. $\Delta H_{1-5}=81\pm3$ G; *g*-factor of central peak 2·004 ±0·001.

6. *Aspartic acid* (Fig. 5 (7)). Poorly resolved quartet or sextet structure. $\Delta H_{\frac{1}{2}}=29\pm2$ G. Distance between adjacent central peaks 15 ±2 G, i.e. less than in other amino acids: *g*-factor of signal centre ≈2·004.

7. *Glutamic acid** (Fig. 5 (8)). Symmetrical signal with clear triplet splitting

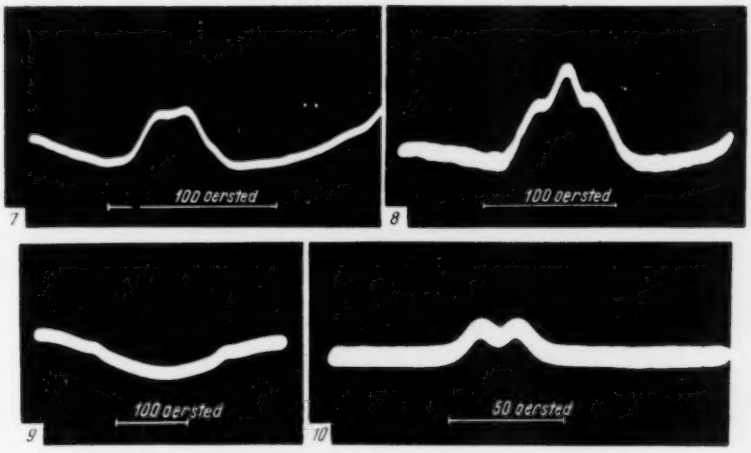


FIG. 5.

(distance between adjacent peaks 18 ±2 G) but two weak side peaks are also present. Similar to Gordy's spectrum. Overall spread 75 G (Gordy: 90 G); *g*-factor of central peak 2·005 ±0·001. Gordy *et al.* may well be right in assuming this a glycine triplet and an alanine quintet superimposed.

8. *Tyrosine* (Fig. 5 (9)). Weak single with a comparatively narrow peak, $\Delta H_{\frac{1}{2}}=9\pm2$ G: *g*-factor of signal centre 2·005 ±0·001.

9. *Cysteine*. No signal.

10. *Methionine*. No signal.

Peptides

11. *Glycylglycine** (Fig. 5 (10)). Strong symmetrical doublet with $\Delta H=16{\cdot}5\pm1$ G (Gordy: ≈12 G); *g*-factor of signal centre 2·0041 ±0·0005.

12. *Glycyl*-l-*leucine* (Fig. 6 (11)). Broad signal with scarcely noticeable quintet splitting. $\Delta H_{\frac{1}{2}}=27\pm3$ G. Over-all splitting of hyperfine structure about 55 G; *g*-factor ≈2·004.

13. *Glycyl-tyrosine* (Fig. 6 (12)). Strongly split symmetrical triplet with very weak end-peaks; $\Delta H_{1^-3}\approx70$ G; *g*-factor ≈2·004.

14. *Glutathione* (Fig. 6 (13)). Weak doublet with $\Delta H=25\pm2$ G; *g*-factor 2·024 ± 0·002, i.e. appreciably higher than for a free electron.

* Denotes preparations studied by Gordy *et. al.* [13].

Proteins

15. *Casein* (Fig. 6 (14)). Very weak narrow signal; $\Delta H_{\frac{1}{2}}=6$ G; possibly doublet splitting; *g*-factor of signal centre $2{\cdot}006 \pm 0{\cdot}001$. A preparation irradiated in air went a yellowy-brown colour and gave no signal.

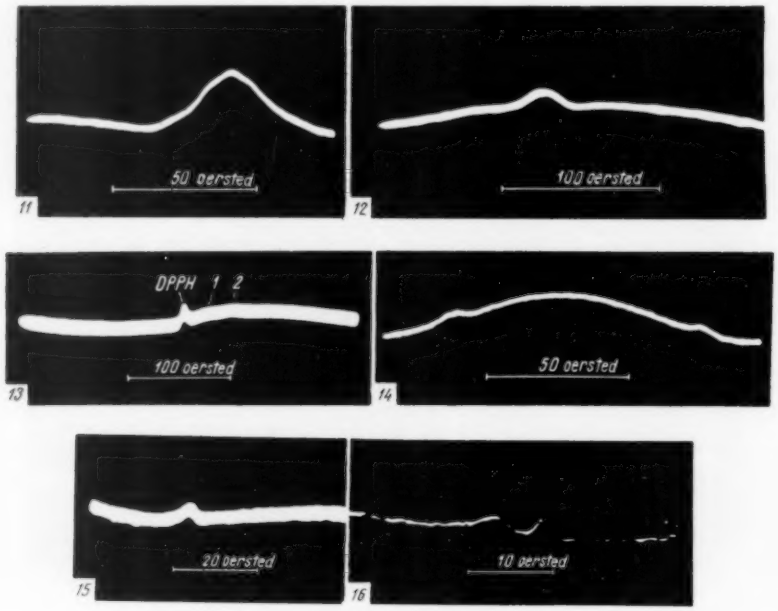


FIG. 6.

16. *Trypsin*. Very weak signal almost at the limit of sensitivity; $\Delta H_{\frac{1}{2}}$ not more than 2–3 G; *g*-factor $\approx 2{\cdot}005$.

17. *Trypsinogen*. As for irradiated trypsin.

Nucleic acids

18. *RNA*. Very weak signal; $\Delta H_{\frac{1}{2}} \approx 10$ G; *g*-factor $\approx 2{\cdot}004$.

19. *DNA*. No signal.

Lyophilized tissues

20. *Rat liver* (Fig. 6 (15)). Weak narrow single signal $\Delta H_{\frac{1}{2}} \approx 2{\cdot}0$ G; *g*-factor $\approx 2{\cdot}005 \pm 0{\cdot}001$. No signal on irradiating in air.

21. *Rat heart muscle*. Signal exactly as above.

22. *Rat spleen*. Signal exactly as above.

23. *Rat lung*. Signal exactly as above.

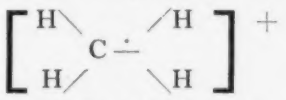
24. *Rat liver*, before lyophilization, boiled once (Fig. 6 (16)). Somewhat wider ($\Delta H_{\frac{1}{2}} \approx 3$–4 G) and stronger displace signal; *g*-factor $2{\cdot}001 \pm 0{\cdot}002$ (diphenylpicryl-hydrazyl peak seen on the right).

After irradiation phenylalanine and tyrosine fluoresce on ultra-violet irradiation. Phenylalanine fluoresces bright yellow, tyrosine orange.

When the irradiated dry amino acids and peptides were stored in air their e.p.r. spectra did not change over several months. The signals from irradiated proteins and tissues vanish in a few days under similar conditions.

DISCUSSION

The e.p.r. spectra of irradiated amino acids and dipeptides give rise to certain deductions as to the radical structures. We agree with Gordy's assumption that the symmetrical quintet in irradiated alanine is given by the radical:

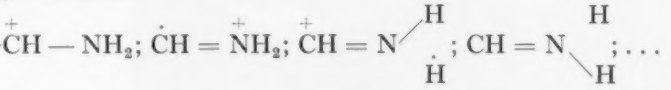


The observed hyperfine splitting (88 G) indicates that the 1*s* orbits of the four hydrogen atoms take up about one-sixth of the unpaired electron (splitting in the free hydrogen atom is at about 500 G). The appreciable line-narrowing on going to phenylalanine, and the absence of a hyperfine structure in this latter, force us to suppose appreciable exchange interaction between radicals trapped in the lattice as well as an aromatic character for the radical. This effect appears even more strongly in irradiated tyrosine.

The valine results reproduce Gordy's if we neglect the somewhat smaller over-all splitting. The assumption that the observed spectrum belongs to two radicals ($C_2H_4^+$ quintet and $C_2H_6^+$ septet) is very likely true.

The quintet signals with anomalously weak end components found in lysine and glutamic acid are due to superposition of the triplet CH_2^+ signal (glycine triplet) on the alanine quintet from $C_2H_4^+$.

The quintet with anomalous intensity distribution found in norleucine may be very tentatively ascribed to $(CH—NH_2)^+$ with an electron distribution described by the following structures:



Such a radical with three hydrogen atoms, two of which are equivalent, should, according to the theory, give a quintet with intensity ratios 1: 1: 2: 1: 1. The small deviations from these values and the broadening of some components may be explained as due to the hyperfine structure not being resolved because the unpaired electron interacts with the ^{14}N, which has a small magnetic moment.

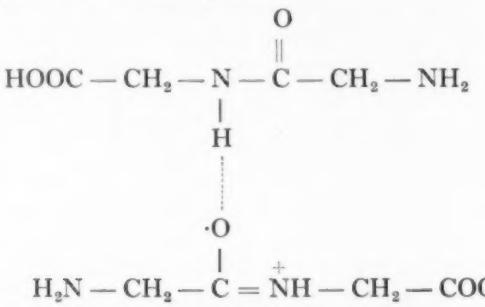
The poor hyperfine structure resolution in aspartic acid prevents its being used to interpret the free radical structure.

It is difficult to explain the absence of e.p.r. spectra from irradiated cysteine and methionine. Although vacuum irradiation was used the spectra were taken in air. The free radicals formed may vanish very rapidly when oxygen is present. Additional work is required to solve this problem, including studies on dose and radiation hardness effects.

To sum up the amino acid results, we may say that γ-irradiation of dry amino acids as a rule leads to stable free radicals giving wide e.p.r. lines with hyperfine structures being formed, which indicates that the unpaired electrons are localized at

particular centres. Even with crystalline aromatic amino acids, in which large exchange effects would be expected, the line-widths are around 10 G.

An analogous picture is observed in the e.p.r. spectra of the irradiated peptides. We, like Gordy *et al.* [13], explain the doublet structure in glycylglycine as being due to the unpaired electron being localized on the oxygen atom involved in an intermolecular hydrogen bond in the crystal:



The observed 16·5 G splitting corresponds to an average distance between unpaired electron and NH-group hydrogen of 1·19 Å, which is reasonably acceptable. The glycyl-*l*-leucine and glycyltryosine also give wide bands with noticeable hyperfine structures. The signal from glycyltyrosine can be interpreted as the glycine triplet superimposed on the tyrosine peak.

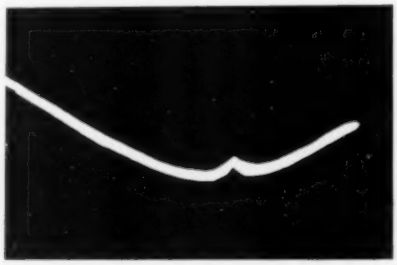


FIG. 7.

Glutathione gives an extremely interesting spectrum. The *g*-factor (2·024) is exactly that for an unpaired electron localized on the S atom [14]. The observed doublet splitting corresponds to an average distance between unpaired electron and hydrogen of 1·01–1·07 Å. Hence it is localized on the S atom: $R-CH_2-\overset{+}{S}-H$. Evidently the γ-ray quantum ejects an electron at some point in the peptide, leaving a hole which is instantly filled by the SH group electron. This effect may be related to the protective action exerted by thioamino acids. Similar effects have been found in keratins [13].

Two special features leap to the eye in the proteins and lyophilized tissues, which contain 60–85 per cent protein. Firstly, the tissues and proteins give 10^3–10^4 times less free radicals than amino acids and peptides at the same dose. Secondly, the signal is not the superposition of e.p.r. spectra for the separate amino acids but a

single peak with $\Delta H_{\frac{1}{2}}$ about 2 G (for casein ~6 G) without hyperfine structure. As the unpaired electron concentration is small (10^{-7}–10^{-8} M/g) the narrowing cannot be attributed to exchange interaction. The translation mechanism [6, 7] has to be assumed, i.e. the electrons are largely delocalized in the protein molecule. The positions and widths of the signals from irradiated proteins and lyophilized tissues are identical with those from tissues frozen and lyophilized while enzyme processes are occurring in them [2].

The spectrum from non-irradiated lyophilized rat liver ($\Delta H_{\frac{1}{2}}$~2 G) is shown in Fig. 7. The sliced preparation was frozen in liquid nitrogen immediately after removal and lyophilized. If the preparation is allowed to stand in physiological saline for several hours prior to freezing to reduce the enzyme process rates by substrate exhaustion, no signal is obtained. Even plunging the liver preparation once in boiling water prior to irradiation results in the signal broadening and shifting, which indicates that the degree of delocalization is reduced. It may be supposed that the unpaired electrons produced in native proteins by the γ-quanta pass into energy levels encompassing the whole protein structure. Many of these fall back, the rest being delocalized about hydrogen bonds lying transverse to the main peptide chains [2]. In fact, in the amino acids and peptides, the signals are due to positive radical ions, i.e. holes with unpaired electrons. The ejected electrons are taken up in lattice traps and give such broad lines that the general background absorption they produce can only be observed in certain cases [13]. Most of the ejected electrons are trapped close to the conduction channels through which the holes circulate.

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It is possible that these concepts could be confirmed by studying the relation of the e.p.r. spectra for irradiated proteins to the previous denaturation. In any case, these results indicate that electronic levels common to the whole molecule exist in proteins. The possible nature of such levels has previously been considered by one of the present authors [2]. We must again emphasize that we are not here speaking of the free migration of a single electron but of the rapid change in the localization of an unpaired charge (electron or hole) along the system of peptide hydrogen bonds transverse to the polypeptide chains. Even if the barrier height is 3 eV (which is undoubtedly exaggerated in this case) the transmission coefficient for electron tunnelling through a barrier width of 2 Å is 0·25, and for 2·8 Å is 0·0056, which is sufficient to ensure the required migration rate.

SUMMARY

(1) The e.p.r. spectra of some irradiated dry amino acids, peptides, proteins and lyophilized tissues have been studied.

(2) The irradiated amino acids and peptides as a rule give characteristic wide e.p.r. lines with hyperfine structures. Some tentative arguments bearing on the structures of the free radicals formed are presented. In glutathione the unpaired electron is localized on the S atom.

(3) The dry protein preparations give very narrow lines of extremely low strength immediately after irradiation. The unpaired electrons here lie in electronic levels common to the whole molecule and are delocalized about the peptide hydrogen bonds running transverse to the main peptide chains.

Translated by J. E. S. Bradley

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THE THEORY OF THE BIOLOGICAL ACTIONS OF RADIATION*

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(*Received* 15 *April* 1957)

ANY cell in a living organism functions under conditions where substrates continually diffuse in and reaction products out, i.e. under "open" conditions. This is also true in relation to the organism as a whole since it is continually exchanging matter and energy with its surroundings. Radiation theory must, therefore, take account of the general features of chemical processes in open systems.

The theory of open systems [1–3] shows that in any such system, which must in principle be a multicomponent system structurally differentiated [3], the steady-state component concentrations must depend at least on the substrate concentrations, on the transfer constants (diffusion, permeability) and on the rate constants for the reactions. Any change in these parameters or in the external conditions (temperature, pressure, etc.) results in a new set of reactant steady-state concentrations. In open systems the diffusion and permeability constants are no less important than the rate-constants, unlike closed systems, so far mainly studied in biochemistry, where the rate constants are dominant, e.g. in enzyme processes. Allowance for transfer as well as rate constants is an inherent feature of the theory of open systems which reflects the indissoluble connexion between the spatial and temporal aspects of metabolic organization. When conditions change, compensatory processes develop which tend to maintain the *status quo*, transitions from one steady state to another frequently involving passage through intermediate maxima or minima in open systems [3]. In the simplest cases the limits of change of a parameter compatible with a stable steady state can be exactly determined; thus in the enzymatic oxidation of ascorbic acid [4]

* Biofizika **2:** 5, 566–572, 1957 [Reprint Order No. BIO 74].

we found the permissible parameter changes to be about 10 per cent. The system is in general unable to maintain its steady state or to restabilize at some fresh level if the changes are larger, and in that case it runs down.

Radiation, like other external factors, acts, in general, to disturb the stationary state, the system being homeostatic over certain ranges of disturbance, but not beyond. However, the essential feature of the biological actions of radiations is that they can cause large physiological effects at low total energy inputs: for example, in mammals the thermal equivalent is that of a temperature rise of only 0·002°C [5]. Apart from this, much of the absorbed energy has no lethal action. Thus, when yeast cells are given 5000 r there are no less than 1000 ionizations per cell but only 10 per cent of the cells die [5]: when bacteria are irradiated with α-particles it often requires the passage of 100 α-particles through each cell to cause the deaths of these cells [6]. Timofeev-Resovskii [7] also remarks on the minute amount of energy dissipated even at high lethal doses and considers that the biological action is related to highly localized processes occurring primarily in microtopographical areas of the object. It is clearly highly important to the theory of radiobiology to elucidate the nature of these microtopographical areas.

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The primary inactivation volumes computed from radiation data are often of molecular dimensions [6, 8]. Target theory [6] is commonly used in such calculations, which on a physical basis gives only a method of calculating the inactivation volume under varying radiation circumstances (differing radiation types, dose rates, etc.), the nature of the volume not being determined. While being insufficient as a general radiobiological theory (since metabolism, physiological state of the cell, effects of various physical and chemical factors on irradiation, etc. are neglected) it is not subject to doubts as to principle within the limits in which it is formally applicable. In some cases target theory has given radiation determinations of molecular weights for enzymes, hormones, viruses, etc., in good agreement with those from other methods [6, 8]; this has been shown to be the case, for instance, in our work on lipoxidase and insulin [9, 10].

We here encounter the central problem of radiobiology—why damage to a few minute areas of molecular dimensions can cause cell death, while damage to hundreds of molecules in the cell may not be lethal; or in terms of the theory of open systems, how damage to a few molecules can cause substantial disturbances in the steady state.

Published work is mainly directed to establishing specific structural changes induced by irradiation in materials such as proteins, nucleic acids, lipids and their corresponding proteins, etc. (see [11, 12] for reviews). In essence, no allowance is made for specifically cellular structures and the complex heterogeneous internal structure of protoplasm; the primary mechanisms of radiation action are frequently dealt with as if the cell were simply a homogeneous solution of the various substances, whereas the complex intracellular structure produces quite special radiation effects distinct from those found in isolated materials: it is this structure which provides the division between true radiobiology and the radiation chemistry of complex molecules.

In current radiobiological theories radiation effects are linked either to damage to nuclear structures (chromosomes) or to microscopic cytoplasmic structures (mitochondria, microsomes, etc.). Since chromosomes are unique cellular structures the

first type of theory [6, 13] explains both cell death with inactivation volumes of molecular order and the genetic effects, but does not explain the physiological changes or many other radiobiological phenomena. Thus, Meisel' [14] states that the fat and lipid metabolism enzymes bound to cytoplasmic structures are as radio-sensitive as are the nucleoprotein ones, and also that radiation disturbance to oxidative phosphorylation undoubtedly involves damage to cytoplasmic structures. The marked relation of number of chromosome aberrations to dose rate, temperature, and type of radiation, and the numerous cases of deviation from exponential relationships, indicate that these changes are probably secondary [13]. The second type of theory [13, 15, 16] attaches particular importance to damage to structurally conjugated enzyme chains in the mitochondria, microsomes, etc. These theories are also of interest, but a great stumbling-block is that the microsomes and mitochondria are so often reduplicated. The much higher radio-resistance shown by polyploid cells [17] (with 2–4 times the normal number of chromosomes) indicates that when tens or hundreds of functionally identical structures are present it is difficult to explain high radio-sensitivity as due to damage to one such structure (if we do not assume the existence of a unique cytoplasmic structure). One attempt, based on open systems theory, to allow for the intracellular structure explains disturbance to the steady state in terms of changes in the diffusion parameter which, however, are caused by damage to only a few molecules.

The cause cannot lie in damage to structures visible under the microscope, formed by thick polymolecular sheaths or films, since damage to a few of these molecules could not substantially alter their permeabilities. Radiation-induced permeability changes have been observed repeatedly (see references in [8]), but only the over-all changes in the distributions of electrolytes or organic compounds between cell and surrounding medium are measured, and these are the result of changes in sorption properties and structure in the cytoplasm, etc., as well as of permeability changes; no precise determinations have yet been made of cell permeability changes on irradiation. In any case, damage to thin intracellular monomolecular or bimolecular interfaces are of principal interest, since damage to only 1–2 molecules can alter the layer permeability.

Protoplasm is very heterogeneous and shows many separate internal phases. At the interface between media of different compositions, orientated molecular layers must arise which are capable of giving the metabolic reactions a spatial organization. This facilitates the existence of regions of widely differing pH (1·5–2·5 units difference) in different areas of protoplasm or of redox potential, zones with differing composition in different areas of protoplasm or of redox potential, zones with differing compositions, etc. [19]. These interfaces sometimes prevent contact between enzyme and substrate and thus retard reactions which can occur when the cell is damaged or dies (autolysis, oxidation of polyphenols, etc.). Bacq and Alexander [13] remark on this, but they deal with permeability changes in mitochondria and microsomes while we devote most attention to the heterogeneity of the cellular protoplast at the molecular or submicroscopic level.

The properties of monolayers of biochemically important materials have been dealt with in several papers and books [20–23]; in this connexion we note the electrically and structurally asymmetric orientated layer, the accentuated structural

mechanical properties, the permeability changes consequent on structure or charge changes, etc. The direct radiation destruction of monolayers has been most studied, and not the permeability changes; but X-ray studies of thin (30–40μ) irradiated films of crystalline polymers show [24] that the formation of only one chemical bond or "bridge" between molecular chains causes structural disordering extending for 10 links or so because the chains are locally drawn closer (1·5 Å instead of 4·5 Å) which substantially alters the layer permeability; the irreversibility of such changes must also be underlined.

In effect, damage to a few molecules at a monomolecular or bimolecular interface makes effective the difference of chemical potential between the large amounts of material in the two adjacent media, just as a small hole in a dyke will cause large amounts of water to reach the same level on both sides. This is of course a far-fetched simile, since the diffusion and permeability changes involve active metabolic processes, but irreparable damage to interfaces must reflect materially on the permeabilities. The key position of the damaged molecules is here due to their being at an interface and not to any specific structural peculiarities they may show; damage to the same molecules present in depth would not cause such large disturbances. The biological amplification of radiation damage may thus be due to the intracellular fine structure. Disturbances of the normal sequence of enzymatic reactions on a substrate, several links being cut out at once, may be more specific than damage to 1–2 enzyme molecules in one of the tens of hundreds of mitochondrial particles.

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The mathematical methods of the target theory are still of value as they cover cases where the primary inactivated volume actually is of molecular dimensions and cases where it is of membrane dimensions (e.g. Zirkle [31] gives $r=800$ Å, $d=0{\cdot}26$–$2{\cdot}1$ Å). Meanwhile it is evident that the inactivated volume is directly involved in metabolism and in the physiological state of the cell, since only in this way can the initial damage spread. When calculating the inactivated volume from the physiological effects of irradiation, it is inevitable that the results should depend on the physiological state of the cell (thus, single-hit and multi-hit curves can be obtained with *E.coli*, depending on the time of incubation in salt solution after irradiation [13]); it is difficult to explain this from concepts of damage to isolated molecules. The primary radiation damage to the interfacial molecules should not depend appreciably on temperature while the subsequent diffusion and enzymatic processes should have high temperature coefficients (Q_{10} ranges up to 3·5 for permeabilities [19]); this explains the two-phase temperature dependence of radiation damage and the possibility of separating the two phases in time by irradiating at low temperatures followed by later heating [25].*

The diffusion and subsequent biochemical processes will naturally develop only slowly in the first period after irradiation, but then become more rapid as metabolism and the intracellular structures coupled to it are disturbed and become observable by direct biochemical and physiological methods; this corresponds with the radiation damage kinetics normally observed [5].

Since the lipoproteins very probably participate in the intracellular molecular

*Analogous effects in proteins and other macromolecules [25] are due to summation of denaturation effects [26] and the large temperature coefficients these have; the mechanisms differ essentially from that giving the *in vivo* temperature dependence of radiation damage.

interface, formation of peroxides on irradiating nucleic acids [27] and unsaturated fatty acids [5] is in complete agreement with our views, particularly if we assume that these peroxides can diffuse in the interfaces and thereby extend the zones showing changes in diffusion properties and the consequent metabolic disturbances (after-effects).

Molecular damage at an interface may occur directly by ionization or excitation by the radiations (ultra-violet light being one such) or via free radicals resulting from the radiolysis of water, etc.: this indicates a direct connexion between the direct and indirect effects (oxygen effect, anoxic effect, etc.).* Radiomimetic substances [11, 13, 29] produce intermolecular bridges which can cause structural distortions in adjacent areas of the thin interfaces, as in radiation cross-linking in polymers [24].

Enzyme system activation [30–33] at doses at which changes in the enzymes or substrates themselves can be neglected is probably explained by the increased contact between enzyme and substrate brought about by damage to the intracellular interfaces. This activation is usually temporary and relates to the transition between steady states via an extremum noted above.

The biochemical disturbances cause anomalous products to accumulate (radiation intoxication [7, 11]) which can diffuse between cells and extend the damage. The part played by the organism's being a single whole and by the interactions between organs in increasing the radio-sensitivity [34] may be explained by the intracellular structures and permeabilities being very probably influenced by nervous and hormonal regulation. The high radio-resistance shown by micro-organisms as compared with tissues from higher organisms is due not only to the absence of the above factors and to the intoxicating products being more readily eliminated, but also to micro-organisms having lower degrees of intracellular organization. As the internal cell structure becomes simpler and less differentiated, less radiation can be localized at interfaces and, in the limit, in a simple homogeneous solution of macromolecules, the radio-resistance should be quite high.

Of course, the above arguments do not reduce the importance of studies on various radiation-induced structural and chemical changes in proteins, nucleic acids, lipids and their related proteins and on structural changes in chromosomes, mitochondria, microsomes, plastids, etc., damage to which may be important sources of biological accentuation of radiation effects. Our task is, while allowing for these factors, to consider also other possible important factors in relation to biological "amplification" of radiation damage, particularly physiological disturbances in cells due to low radiation doses. In doing this we bear in mind the close connexion between radiation damage to intracellular structures and interfaces and the changes in diffusion parameters in the steady state with their consequences in metabolic disturbances. It was shown above that the basic problems of radiobiology are elucidated by adopting these views. The importance of studying radiation-induced permeability changes in various types of monolayer is thereby emphasized: this topic is ripe for experimental study. But at present we may suppose that this factor may play an important part in the biological amplification of radiation damage, and hence in radiobiological theory.

* The HO_2 and OH radicals may give redox potentials of $E = 0{\cdot}8$–$0{\cdot}95$ V [13]; penicillin, also an oxidant ($E = +0{\cdot}3$ V [28]) shows a number of general resemblances to radiation in its action on bacterial cells (giant-cell formation, identical gradation in protection by cysteine, glutathione, thiourea, etc.).

SUMMARY

The conditions in which the primary radiation-inactivated volumes of molecular dimensions can disturb the steady state of the cell via changes in the diffusion parameters are elucidated from concepts in the theory of open systems. It is shown that the disturbances can occur via damage to intracellular molecular interfaces. The main problems in the primary mechanism of biological radiation effects are considered from this viewpoint.

Translated by J. E. S. BRADLEY

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THE MIGRATION MECHANISM OF RADIATION PROTECTION*

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(*Received* 25 *April* 1957)

One of us has earlier published an hypothesis concerning the primary mechanism of the biological action of radiation [1], the essence of which is as follows. The energy absorbed either directly or indirectly on irradiation damages the system in the course of migrating either within or between the protein or other macromolecules. Some of the damage responsible for the physico-chemical radiation after-effects is retained in the macromolecules as long-lived electronically excited states; it does not escape and in time produces additional effects. We suppose that this damage can be removed by intermolecular energy migration to some protective agent which forms a complex with the macromolecules. This paper briefly presents work designed to test this idea.

The normal protection effect is ascribed to concurrent reaction between the object and protector, on the one hand, and radicals produced by radiolysis of water on the other. To eliminate this mechanism we studied protection mainly on after-effect phenomena.

We used an actomyosin complex formed *in vitro*. The degree of complexing, as estimated from the viscosity of the actomyosin gel, can be adjusted by varying the component ratio or by using globular actomyosin (A_g) instead of the fibrillar (A_f). The myosin was made by Lyubimova's method from rabbit muscle by 0·5 M KCl extraction. It was mixed in known ratios in solution with actin (Straub) solutions of the same concentration (4 mg protein/ml).

The protein solutions were X-irradiated (80 kV, 20 mA, 0·155 mm Al). The damage to the myosin was estimated from the ATPase activity. In all cases 75 kr doses were used, 35 per cent of the activity then remaining.

After-effects exist in this system, as additional thermal treatment studies show. The irradiated protein differs sharply from normal in heat-stability. All operations with the myosin prior to incubation with ATP were therefore conducted in the cold.

Thermal inactivation related to radiation after-effect has a sharp temperature threshold (with a step of 5–10°C) and the time-dependence is exponential with a temperature-dependent exponent (Fig. 1).

The non-irradiated myosin (M) activity is independent of actin complexing ($MA_f/M = 1{\cdot}0 \pm 0{\cdot}03$; $MA_g/M = 1{\cdot}0 \pm 0{\cdot}04$). If previously irradiated myosin [M]† is complexed with the same actin and the two components at once separated (by ATP in the activity determination) the activity is found to have changed from 35 per cent to 50–60 per cent of the non-irradiated. Thus, the very act of complexing protects myosin from inactivation (Fig. 2).

† In Figs. 1 and 2 the irradiated materials are enclosed in circles.

* Biofizika 2: 5, 573–575, 1957 [Reprint Order No. BIO 75].

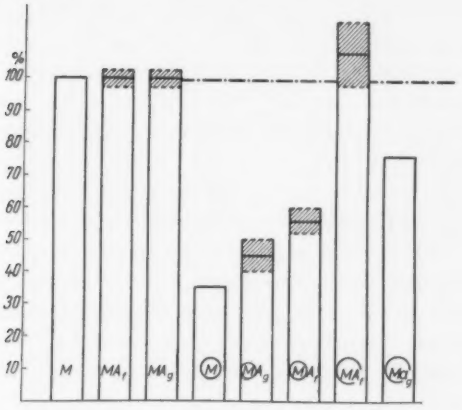

FIG. 1.

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The protection depends on the extent of complexing. Globular actin has a weak effect ($[M]A_g/[M]=1{\cdot}30\pm0{\cdot}15$), fibrillar being more active in all cases; on average $[M]A_f/[M]=1{\cdot}62\pm0{\cdot}10$. The effect depends on the myosin–actin ratio, as judged by the viscosity (i.e. degree of complexing). Thus, the viscosity fell by a factor 1·5 on going from $M/A_f=1$ to $M/A_f=4$, and the protection effect by a factor 1·4. The myosin is activated stepwise at the instant of mixing. The difference is retained on storage and does not diminish. If the irradiated myosin is stored in the cold it can be activated by complexing with actin at any time up to several days after irradiation.

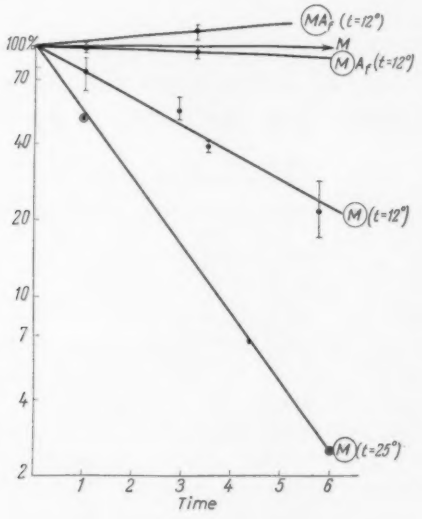

FIG. 2.

When the $[M]A_f$ complex has split up, either spontaneously by prolonged cold storage, or under the action of ATP, the myosin appears activated.

When the irradiated myosin is actin-complexed not only is it activated but also the cause of after-effects is removed. Thus, thermal inactivation of the protected myosin occurs as in the non-irradiated substance, being quite different from that in the unprotected myosin (Fig. 1).

Marked protection and simultaneous after-effect elimination were also observed when the actin was present during the irradiation. The protective actions of A_f and A_g were compared. The medium and protein compositions of the solutions were as before, the sole difference being the degree of complexing between components. This eliminates possible concurrent protection mechanisms (radical capture).

At high degrees of complexing (relative viscosity of MA_f solution 8–9) the myosin was completely protected: $[MA_f]/MA_f=[MA_g]/M=1{\cdot}08\pm0{\cdot}10$. On average, A_g had less effect: $[MA_g]/[MA_f]=0{\cdot}8$ (Fig. 2). In experiments where the viscosity of the MA_g solution was $\sim$2 the residual myosin activity was raised by only 10–20 per cent, while at viscosity 3–4 the protection rose sharply (only 10–20 per cent activity loss in co-irradiation). The irradiated A_g-myosin complex showed post-irradiation thermal inactivation as for the non-irradiated material (Fig. 1).

These results confirm our hypothesis that there is a migration mechanism of radiation protection.

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SOME REGULARITIES IN THE RETARDATION OF THE FIRST DIVISION IN YEAST CELLS ACTED ON BY ^{60}CO γ-RAYS*

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As we have already shown [1], yeast cells irradiated with ^{60}Co γ-rays form macrocolonies differing much from normal when cultured on wort-agar. Firstly, the mean rate of colony formation is linearly related to the reciprocal of the dose from $12–74\times10^3$ r. Granat and Sherman [2] observed an analogous effect on growing X-irradiated *Torulopsis utilis* on a liquid medium. Secondly, these macrocolonies fall into at least two groups with differing growth rates. Moreover, an X-ray dose which inactivates 99 per cent of the yeast cells retards division in only 50 per cent, while cells incapable of producing numerous descendants can be seen to be dividing normally [3]. However, the relation between the growth of macrocolonies produced by γ-irradiated

* Biofizika **2:** 5, 576–580, 1957 [Reprint Order No. BIO 76].

yeast cells and the action of radiation on the first division remains unclear. Here we attempt to elucidate this problem.

METHODS

The diploid yeast *Saccharomyces vini*, Megri-139B strain, was used. The methods of preparing the suspension and of irradiation have already been given [1]. The γ-ray source was a GUT-Co-400 unit (half-strength source) giving 820 r/min at 5·5 cm. Yeast cultures 48 hr old and containing not more than 0·1–0·2 per cent dividing cells were irradiated. The cell concentration in the irradiated suspension was about 6×10^7/ml.

After irradiation 0·4 ml portions of the suspension were placed in tubes and rapidly mixed with equal volumes of unfermented beer wort of 10° strength. The tubes were incubated at 30°C for 6 hr. Fermentation kept the cells suspended throughout the experiment and the small volume used ensured that aeration was adequate.

The suspensions were sampled for smear preparation every 30 min. Cell division was blocked by adding formalin. The smears were dried, treated with 96 per cent ethanol for 3–5 min and stained with gentian violet. The fraction dividing was determined by counting on some 1000 cells at ×900 at 30–50 points in the smear. An un-irradiated suspension was used as control. This method gave high accuracy and reproducible results. The differences between duplicates did not as a rule exceed 2–3 per cent, and between repeat experiments 10–15 per cent.

RESULTS AND DISCUSSION

The relative dividing cell contents during the 6 hr incubation following irradiation with ^{60}Co γ-rays at various doses are given in Table 1. The experiments at each dose were repeated 2–4 times.

TABLE 1. PERCENTAGE CONTENTS OF DIVIDING CELLS DURING 6 HR INCUBATION AT 30°C FOLLOWING IRRADIATION WITH ^{60}Co γ-RAYS

Dose (kr)	Incubation time (hr)										
	0	1	1·5	2	2·5	3	3·5	4	4·5	5	6
Control	0·2	0·1	1·5	11·2	32·5	66·1	77·9	64·8	58·1	61·2	69·1
12·3	0·1	0·2	0·1	0·1	7·2	25·3	54·8	79·1	90·8	95·1	85·0
24·6	0·2	—	—	0·1	4·3	20·7	43·1	72·3	91·3	98·2	88·8
49·2	0·2	—	—	0·2	3·7	17·1	38·0	64·6	—	95·7	93·2
98·4	0·2	—	—	0·1	—	11·3	37·4	64·1	—	88·1	91·0
295·2	0·1	—	—	0·1	1·2	11·1	36·6	53·5	—	69·8	—
307·5	0·2	—	—	0·1	1·2	7·9	19·0	24·1	—	28·8	28·2

The maximum contents occur at $3\frac{1}{2}$ hr from the start of incubation in the controls. Fig. 1 (curve *A*) shows the percentage division vs. dose for the same time on a semi-log plot (control = 100 per cent). The survival curve (*S*), determined in the usual way by colony counting, is shown for comparison. Fig. 2 shows the division rates for irradiated and non-irradiated cells during 6 hr incubation.

The dividing cell content falls rapidly at doses having little effect on the capacity for unrestricted division (Fig. 1(*A*)), falling to 60 per cent even at LD 10 per cent.

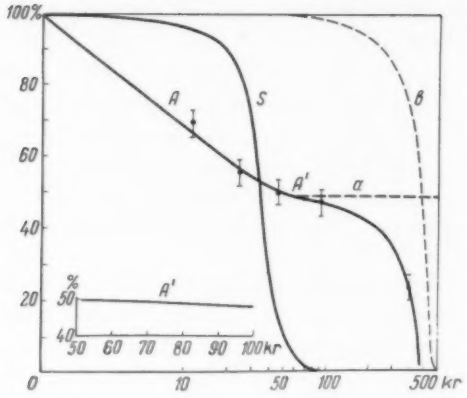

FIG. 1. Effect of radiation on the first division. The first division retardation (curve *A*) and its components: division initiation lag (*a*) and immediate inactivation (*b*). The survival curve (*S*) is shown for comparison. Abscissa: dose in kr, log scale.

Further increase to LD 99 per cent has only a slight effect. Sarachek *et al.* [3] observed similar effects with X-irradiated diploid yeast cells. The second sharp fall only occurs at doses several times the absolute lethal value.

To distinguish the processes responsible for the shape of Fig. 1(*A*), consider Fig. 2. Curve (1) shows that budding in non-irradiated cultures begins at $1\frac{1}{2}$ hr after placing in the incubator and rapidly spreads to most cells, reaching a maximum of 78 per cent at 2 hr. The fall is explained by the buds splitting off, and the second rise by the second cycle of division.

The shape of the curve is due to the yeast cell population being inhomogeneous. The shape is determined firstly by the content of cells capable of dividing, secondly by the mean division cycle time and thirdly by the degree of synchronism between cells. The first two determine the position of the maximum and the third the steepness of the curve.

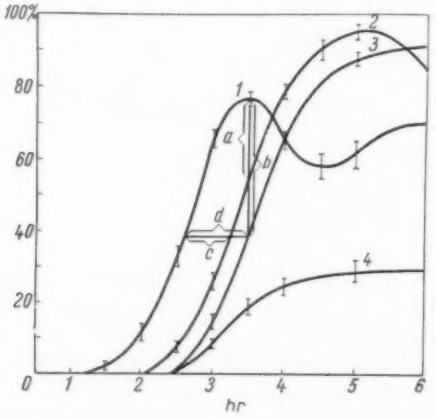

FIG. 2. Division at 30°C. (1)–control, (2)–12·3 kr irradiated, (3)–98·4 kr, (4)–393·6 kr. Abscissa: hr of incubation.

Fig. 2 (2, 3 and 4) show the behaviour of cells irradiated at 12·3, 98·4 and 393·6 kr respectively. A dose of 12·3 kr has no material effect on the content of cells capable of dividing, nor on the synchronism. Curve (2) is parallel to (1) over the range 0–78 per cent. Thus, this dose only temporarily retards division, the delay being about 36 min. The higher maximum in (2) as compared with (1) is due to a second radiation effect—delay in splitting off the new buds, and possibly reduced new bud-growth rate.

A dose of 98·4 kr also has no material effect on the synchronism. The maximum height and delay in division are not substantially different from those for 12·3 kr. Curve (3) is only 6 per cent lower than (2), which is due to the production of cells incapable of dividing, at least during 6 hr incubation at 30°C. This is the third type of radiation effect on the first division in yeast.

At 393·6 kr, as the start of curve (4) shows, there is no further increase in delay. But the synchronism and rates of budding are much changed, which affect the slope, and the number of cells capable of dividing is also changed which affects the position of the maximum.

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Let us now consider the actions of radiation on division delay and on the capacity of cells to reproduce separately.

In 1931 Wyckoff and Luyet [4] found that yeast, previously X-irradiated, when cultured on agar plates gave numerous typical two-cell colonies. This they explained by supposing ionizing radiation unable to block division if this had begun immediately before the irradiation. But in our suspensions the vast majority (98 per cent) of the cells had been physiologically at rest for the previous 24 hr, so the cells could not be in process of division either before or during the irradiation. This is confirmed by the smears prepared immediately after irradiation, the control and irradiated suspensions containing not more than 0·1–0·2 per cent dividing cells (see Table 1). But almost all the cells irradiated at doses below 100 kr budded when kept at 30°C in the nutritive medium for 2–3 hr. The first division is thus highly radio-stable.

Fig. 2 shows that at doses less than 90 kr the lesser number of cells dividing at $3\frac{1}{2}$ hr (sections *a* and *b*) is almost entirely due to the delay, represented by the lengths *c* and *d*, where $a/c=b/d$. Thus, at these doses the height of the delayed growth curve (Fig. 1(*A*)) is entirely determined by the delay in the first division. The figure also shows that irradiation cannot delay the start of division by more than about 1 hr, as at 98·4 kr. Saturation sets in above this dose, as in other cases [5, 6], the ionizing radiation having no further effect. The accumulation of some unknown damaging factor is also complete, this factor inactivating the cells during or immediately after the irradiation. This inactivation becomes appreciable at doses a factor 10 greater than the LD 50 per cent (Fig. 2(4)). At these doses the ordinate of Fig. 1(*A*) depends on the sum of the division delay and irreversible inactivation effects.

The retarded division curve can thus be split up into two components, curve (*a*) representing the delay in the commencement of division, and (*b*) showing the percentage of cells which have irreversibly lost the power to divide. The delayed inactivation appears as the failure to form macrocolonies and determines the shape of the standard damage curve, while the other effects can be termed immediate inactivation.

The data show that the growth laws found in macrocolonies deriving from irradiated yeast cells cannot be explained by radiation effects appearing in the first

post-irradiation division. In fact immediate inactivation (Fig. 1(*b*)) occurs at doses greatly exceeding those required to cause cells to lose the capacity to divide without limit. On the other hand, the time lag in the start of division and the way this varies with dose are quite different from the growth-rate drop found in the macrocolonies deriving from cells which have not been inactivated [1]. The ^{60}Co γ-rays evidently produce an early radiation after-effect on the first division.

CONCLUSIONS

The results from studies of ^{60}Co γ-ray effects on the first division in 48 hr yeast cultures are as follows:

(1) Three types of multiplication disturbance are produced: (a) reversible delay in the commencement of division, (*b*) irreversible immediate inactivation, (*c*) delays in growth and budding-off.

(2) The delay in division initiation rises rapidly with dose to a certain limiting value (1 hr at 50 kr), saturation occurring thereafter.

(3) Doses of several hundred kr are required to produce immediate inactivation.

(4) The regularities in the action of radiation on macrocolony growth are not direct consequences of effects on the first division.

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QUANTITATIVE ASPECTS OF THE RECOVERY PROCESSES IN ORGANISMS AFTER GENERAL IRRADIATION*

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(*Received* 13 *February* 1957)

THE laws governing the recovery processes in irradiated organisms have long been the subject of attention from radiobiologists. In the early work [1–7] the production of erythema in patients given therapeutic local irradiation served as a test of the biological action of radiation. In some cases [1, 5, 6] it was concluded that the rates of the processes varied exponentially with the time elapsed since irradiation. In later work animals given whole-body irradiation were used, various types of radiation being employed: β-particles [8], γ-rays [9] and X-rays [10]. But the results gave no laws of recovery process development after whole-body irradiation. Our work has

* Biofizika **2:** 5, 581–588, 1957 [Reprint Order No. BIO 77].

been directed to the quantitative aspects of these processes, over-all estimates being used (survival rates).

METHODS

Male white mice 2–2½ months old were used, of weight 20–25 g. Altogether, 1024 were used. To determine the extent of recovery from general irradiation fractionated (two-shot) irradiation was used. To obtain a given effect, e.g. 50 per cent deaths, with fractionated doses, larger over-all amounts are required than with one-shot doses. This difference is due to the recovery processes operating between irradiations, so if the second irradiation is given at various time-intervals after the first, various LD_{50} values will be found, which values can be used to describe the processes.

Our experiments were carried out as follows. The first doses were sublethal (250 r), the second being given 1, 2, 4, 7, 14, 21 and 28 days later and being so graded that the $LD_{50/30}$ could be calculated for each time interval. The $LD_{50/30}$ for single-shot irradiations was determined simultaneously. The two sets of $LD_{50/30}$ values were then compared, the differences being used to describe the recovery. The process rates were evaluated from aspects such as the shape of the mortality curve as functions of dose and time of death. The irradiation conditions varied somewhat from series to series: voltage 150–180 kV, current 6–10 mA, focal distance 22–23 cm, filters 0·5 mm Cu + 0·75 mm Al, dose rates 33·3–54·0 r/min. Usually 11–12 mice were placed in special Plexiglas containers for irradiation. Survival rates were recorded for 30 days after the second irradiation.

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RESULTS

Table 1 gives the results, namely: number of animals used, number dying and mean survival time of those dying.

These data were used to derive mortality-dose relationships for one- and two-shot irradiation. The $LD_{50/30}$ for single-shot cases for the two-dose experiments were computed by probit methods. In all cases the probabilities (p_g) of the experimental results corresponding with those expected from theory were quite high ($>0{\cdot}1$) (Table 2).

As stated above, the differences in the $LD_{50/30}$ for one- and two-shot doses can be used to specify the "recovery" quantitatively. This parameter is somewhat arbitrary because the recovery processes are judged solely from the radio-sensitivity changes.

The "recovery" at any time after a dose of 250 r can be expressed as a percentage of the damaging action of this dose (taken as 100): it was computed from the following formula:

$$R = \frac{LD_{50/30}\text{ (two-shot)} - LD_{50/30}\text{ (one-shot)}}{\text{Initial dose}} \times 100. \qquad (1)$$

When recovery from the radiation damage is complete $R=100$ per cent. If no recovery occurs $R=0$. The degree of recovery is thus specified by the 0–100 per cent range.

Table 2 and Fig. 1 show that the rates and natures of the recovery processes vary with time after irradiation. The 30 days after irradiation can be divided into three periods differing quite clearly in their final results. In the first 48 hr (period I) R is

TABLE 1. EFFECT OF DOSE ON SURVIVAL TIMES OF MICE

Initial dose (r)	Interval between doses (days)	Second dose (r)	Number of animals used	Dying		Mean survival times of those dying (days)
				Number	Per cent	
0	0	300	70	8	11·4 ± 3·80	15·53
		450	70	31	44·4 ± 5·95	11·88
		600	70	67	95·8 ± 7·59	9·96
		850	59	59	100·0	6·77
250	1	150	46	31	67·4 ± 6·53	18·25
		300	57	38	66·7 ± 6·24	10·28
		450	46	41	89·2 ± 4·56	10·54
		600	45	45	100·0	5·7
		750	35	35	100·0	4·93
250	2	150	24	12	50·0 ± 3·22	20·12
		300	24	17	70·9 ± 2·95	11·73
		450	24	23	95·8 ± 4·08	7·62
		600	24	24	100·0	5·71
		750	24	24	100·0	4·20
250	4	150	24	6	25·0 ± 8·85	16·50
		300	24	14	58·3 ± 3·17	14·98
		450	24	20	83·4 ± 7·58	8·96
		600	24	24	100·0	8·08
250	7	300	24	5	20·9 ± 8·30	11·25
		450	23	17	73·8 ± 9·16	8·48
		600	23	16	69·6 ± 9·60	5·81
		750	24	24	100·0	3·66
250	14	300	23	4	17·4 ± 7·81	16·25
		450	24	5	20·9 ± 8·30	14·08
		600	23	20	87·5 ± 6·89	5·98
		750	22	17	77·3 ± 8·91	4·11
250	21	300	35	5	14·3 ± 5·90	21·60
		450	33	25	75·7 ± 7·46	10·57
		600	34	31	91·2 ± 4·69	8·12
		750	31	30	96·7 ± 3·21	8·72
250	28	300	22	5	22·4 ± 8·89	21·30
		450	22	12	54·4 ± 3·36	14·33
		600	22	22	100·0	7·18
		750	21	20	95·2 ± 4·64	6·50

TABLE 2. RADIO-SENSITIVITY CHANGES IN MICE AFTER WHOLE-BODY 250 r DOSES

Initial dose (r)	Interval between doses (days)	$LD_{50/30}$ for second dose (r)	p_g	True $LD_{50/30}$ for two-dose irradiation (r)	Relative recovery, per cent (from formula (1))
0	0	431·8 ± 1·026	0·10	431·8 ± 1·026	—
250	1	139·8 ± 1·180	0·30	389·8	—16·80
250	2	162·2 ± 1·140	0·75	412·2	—7·88
250	4	236·7 ± 1·111	0·80	486·7	+21·96
250	7	402·7 ± 1·068	0·40	652·7	+88·36
250	14	492·9 ± 1·080	0·20	742·9	+124·44
250	21	387·3 ± 1·012	0·60	637·3	+82·20
250	28	397·0 ± 1·178	0·75	647·0	+86·04

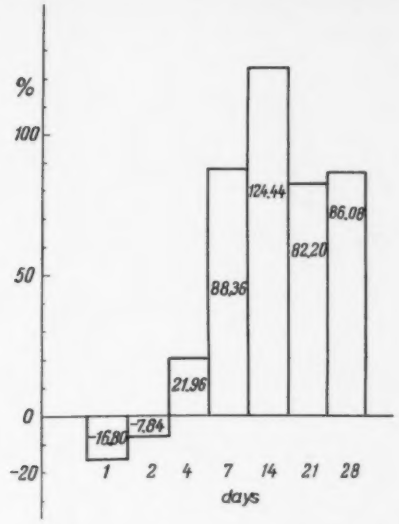

FIG. 1. Integral recovery of mice on whole-body irradiation at 250 r. Abscissa: time after irradiation; ordinate: relative recovery (per cent).

negative, being −16·8 per cent at 24 hr and −7·8 per cent at 48 hr. These results show that further damage probably develops in this period from that arising during irradiation. In other words, the recovery processes occurring in the early post-irradiation period are such as not to be decisive in the integral (viability) response. The predominance of degenerative changes in the initial period of radiation reaction increases the radio-sensitivity in the first 48 hr post-irradiation. At 24 hr 139·8 r suffice, at 48 hr 162·2 r suffice to produce 50 per cent deaths when 250 r doses are given initially. The $LD_{50/30}$ values for irradiation repeated at 24 and 48 hr are less than those for single-shot doses (Table 2).

Further study of these results shows that at day 4 (period II) recovery begins to predominate appreciably over degeneration, R becoming +21·9 per cent. Up to day 14 high recovery rates are found, 88·6 per cent of all damage being repaired at day 7 with $LD_{50/30}$ for repeat irradiation on day 7 being 402·7±1·1, i.e. close to the value for single-shot doses (431·8±1·0). At day 14 mice become super-resistant, R being over 100 per cent (124·4 per cent). Thus not only is recovery complete at day 14, but some irradiation immunity has developed, the $LD_{50/30}$ for repeated irradiation being higher than for one-shot doses at day 14 (Table 2).

For days 21–28 (period III) R is close to the complete recovery value, but no super-resistance is seen. The $LD_{50/30}$ is here somewhat below the single-shot value.

Another index derived from the mortality-time curves at different doses was used in addition to the $LD_{50/30}$ values. The change in death distribution as a function of dose was determined from the slopes of the mortality curves or of the corresponding regression lines (computed by probit methods). After transforming the S-shaped curves into straight (regression) lines, b in $\bar{y}=z+b(x-\bar{x})$ is the slope of the regression line. Fig. 2 shows that the regression line for two-shot irradiation gives a value of b

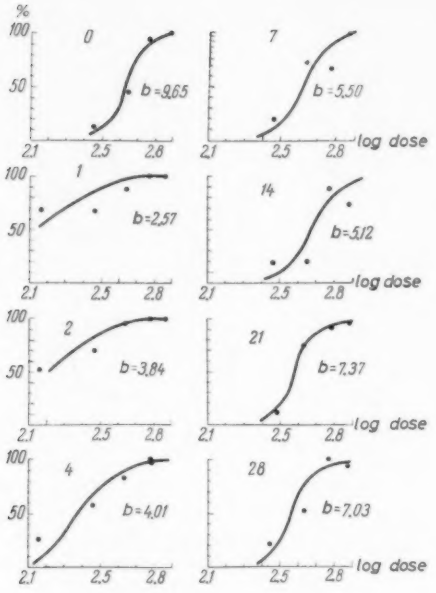

FIG. 2. Change in the shape of the mortality curve and in the slope of the regression line, b, vs. interval between irradiations. Ordinate–per cent of animals dying.

which differs substantially from that for single-shot irradiation; b gradually increases with the interval between doses and approaches the single-shot value. Thus, b is a good quantitative index of the recovery processes. It may be that b is exponentially related to time after irradiation.

The third index is the time distribution of post-irradiation deaths. The time of death is known to be strictly related to dose [11]. It has also been shown that the mortality distribution depends on the strain of mice as well as the dose [12]. We used a comparison of the times to death of singly and doubly irradiated animals to derive an index of the recovery processes. Fig. 3 shows that the death distribution, and also the peak period for deaths, are altered in repeated irradiation as compared with single-shot. When the second dose was 300 r the reaction (day 28) to the second irradiation was analogous to that to the first. At the higher second doses (450 and 600 r) the return to the death distribution typical of single-shot irradiation was slower. This may be because the large doses reveal incomplete recovery more clearly.

DISCUSSION

The recovery process on whole-body irradiation is complex and essentially different from that demonstrated earlier for recovery from local irradiation. This is particularly so as regards the first 48 hr after irradiation. Thus, when skin erythema was studied [4–6] it was found that recovery was most rapid in the first 6 hr after irradiation since some 69 per cent recovery had occurred in 24 hr. The rate of recovery thereafter fell, recovery being 78 per cent at 48 hr.

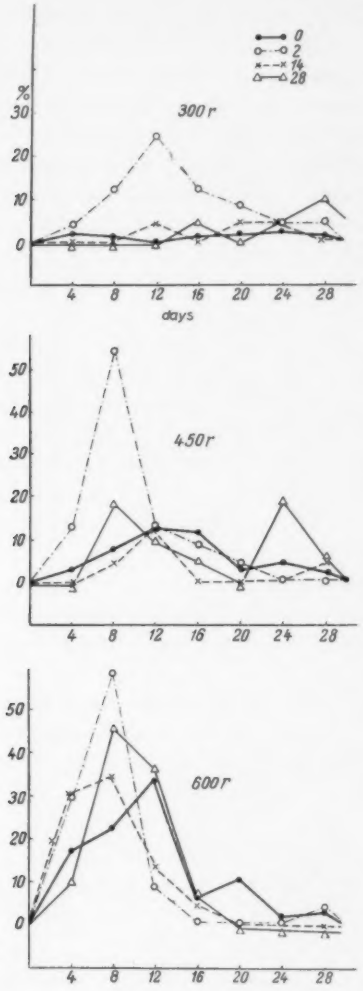

FIG. 3. Death distribution curves as functions of dose (300, 450 and 600 r) on repeated irradiation, and of interval between irradiations (0, 2, 14 and 28 days). Abscissa: time after irradiation; ordinate: per cent of animals dying.

The conflict between our data and those for skin erythema is probably due to the essential difference between the experiments. The erythema was produced by local irradiation and hence the recovery evoked locally was studied, whereas in our experiments whole-body irradiation was used and the recovery laws were studied on integral indices such as survival. Estimates of this kind take no account of recovery processes which have no material effect on viability (e.g. recovery from erythema).

In those experiments in which the time course of recovery was studied on laboratory animals given whole-body irradiation the early post-irradiation period was

either completely neglected [8] or else the results were so obscure as to be omitted from the discussion [10]. Only Curtis [9], who used whole-body irradiation and viability criteria also, obtained data which, as it were, contradict ours. He observed 50 per cent repair of all damage at 2 days after γ-irradiation, recovery being complete at 5 days. The discrepancy may be related to the magnitude of the initial dose. As we have noted above, the viability depends on the balance between degenerative and reparative processes. The lesser destruction produced by the lower doses must evidently enhance the chances of recovery processes. It is very likely that when the first dose is large enough the radio-sensitivity would be raised for several days after irradiation. This phase of increased sensitivity could not appear clearly at low initial doses.

The increased radio-resistance at 14 days after the first irradiation is of particular interest. Similar effects have been observed previously by other workers [8, 13–15]. But there are also data which do not indicate increased resistance in animals (rats and guinea-pigs) after previous irradiation [16, 17]. Such apparently contradictory results can be reconciled if we bear in mind the differences in the experimental conditions used. The initial dose, the timing and magnitude of the second dose, and the experimental species, are so different that it would be better to say the results were incommensurable rather than contradictory.

The increased radio-resistance at 14 days after the first irradiation at 250 r can be explained as overcompensation. The overcompensation, like all recovery processes, depends on the initial dose, so we may suppose it to occur at differing periods after the first irradiation.

Our data thus indicate considerable radio-resistance changes after whole-body irradiation. The changes are phasic in type and are related to specific laws of recovery. Our data on the complex nature of the recovery processes are confirmed by Mole's recent work [18].

CONCLUSIONS

(1) The sensitivity to a second X-irradiation is found to vary with the time after a first dose of 250 r in mice. The changes are phasic in type and are the resultant of degenerative and recovery processes.

(2) The radio-sensitivity is higher in the first 2 days after 250 r. The degenerative processes evidently are progressive and not balanced by recovery ones in this period.

(3) The radio-sensitivity is normal between the fourth and seventh post-irradiation days, indicating that the recovery processes operate rapidly.

(4) A tendency to super-resistance is seen at 14 days after 250 r, which tendency subsequently dies away (by the twenty-first–twenty-eighth day).

(5) As well as the LD_{50}, the change in mortality curve shape, and the time distribution of deaths, can serve as good quantitative indices for the recovery processes.

(6) The precise magnitude and period of radio-sensitivity change depend on the radiation dose.

Translated by J. E. S. BRADLEY

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SOME STRUCTURAL AND CHEMICAL PHENOMENA IN THE STIMULATED NEURONE*

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(*Received* 25 *June* 1957)

IN considering problems of the origin and transmission of nervous excitation, it is essential to bring together in a single theoretical framework the metabolic, electric and structural processes taking place in the nerve tissue. Most study has been devoted to the electrical processes which reflect to some extent the processes of metabolism in the living cell. It has been demonstrated that the biopotentials depend on temperature, on oxygen content of the environment and that they are suppressed when certain enzyme systems and links are blocked. Thus, a no less important aspect of nervous processes is presented by the chemical reactions of metabolism and the structural changes which may be supposed to be directly connected with these. In this connexion, electrical activity is only one link in the long chain of physical and chemical processes connected with function. The data on metabolic changes, and even more so on structural ones so far available are few, but they are of such fundamental importance that they call for discussion at the present time.

Even during the last century numerous attempts had been made to discover some morphological changes in the body of the nerve cell and its processes during functional

* Biofizika **2:** 5, 589–601, 1957 [Reprint Order No. BIO 78].

activity and under electrical stimulation. The nerve cells were, however, studied as fixed tissues, i.e. tissues affected by the action of a fixative which was itself an irritant of great strength. According to some authors, after electrical stimulation lasting many hours, or after prolonged muscular exercise, a decrease in chromophil matter was observed, and also changes in size, shape and internal structure, in the nerve cells [1–5]; other authors found no changes at all.

There were two weak points in histological studies of this kind:

(1) Prolonged electrical stimulation could have a strong traumatic action on the nerve cells; this is all the more likely as morphological changes were not apparent when stimulation was weaker and less prolonged.

(2) The action of fixing agents evokes posthumous changes in nerve cells which lead to considerable variation in structure [6], and these changes develop over several hours after fixation.

On this basis one may have doubts about the results of one histological study of recent years [7], whose authors observed a decrease in the Nissl substance in the nucleus of the hypoglossal nerve of a cat only after 8 hr of stimulation by electric current, the change becoming apparent only 12–60 hr after the stimulation. In a subsequent study, whose authors provided against possible mistakes due to the processing of the fixed tissue, there was failure to discover any change in the Nissl substance in the nerve cells after prolonged stimulation [8].

The question of the presence or absence of morphological changes in a nerve cell subjected to prolonged stimulation has in fact given way, in the above-mentioned works, to a question on another plane—that of the susceptibility or non-susceptibility of nerve tissue to damage.

While early research workers considered the neurone to be an exceedingly sensitive cell, easily damaged by electric current, anoxemia, toxins and other factors, those of more recent years have sought to change this view, representing the neurone as a system with much greater resistance.

A certain modification of the histological method has made possible [9, 10] the discovery of indubitable change in the proteins and nucleoproteids in the cytoplasm of ganglionic cells in connexion with motor and sensory functions.

Cytochemical analysis, carried out on stained sections of nerve cells by measuring the absorption of ultra-violet monochromatic radiation, gives the possibility of establishing changes in the content of nucleic acids and nucleoproteids. The use of this method has shown a considerable decrease in the content of nucleic acids and nucleoproteids under acoustic stimulation in the ganglionic cells of the cochlea of the guinea-pig, and this decrease was reversible. The same changes were shown in spinal motor cells after intensive muscular work or after 5–10 min of electrical stimulation. The decrease in content of proteins and nucleic acids is to a level of one-third or one-fifth of what it was previously, with subsequent restoration in the course of 48 hr.

These facts provide a basis for the supposition that alterations in protein metabolism in the nerve cell are a normal process, connected with changes in functional state. Change in metabolism proves to be a more sensitive and trustworthy criterion than the morphology of the cell.

One would expect the link between structural and metabolic processes in the cell to be on the molecular–micellar level. From this point of view, a more sensitive test

of the functional state of the cell would be given by changes in the sub-microscopic complexes. Here one comes up against the problem of studying *in vivo* the dynamics and connexions with function of sub-microscopic structures. This problem is being steadily worked on by G. M. Frank, and finds experimental treatment in the works issuing from the laboratory under his direction.

At the present time there are already data establishing the close dependence of functional and structural submicroscopic processes on specialized cells such as those of nerves and muscles.

Various methods were used for observations which revealed changes in the physical and chemical states and sub-microscopic structure of the axoplasm when the nerve was stimulated [11–13], and sub-microscopic rearrangements in muscle corresponding to different functional states [14].

The study of living structures must bridge the gap between molecular–micellar complexes and metabolism. With this aim in mind, X-ray analysis and electron microscopy can be supplemented by other methods which make observations *in vivo* possible: birefringence, light-scattering at various angles, study in the dark field, ultra-violet spectrophotometry, vital staining, etc.

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The intensity of metabolic processes in nerve cells is comparatively greater than that in their specialized processes, the axons. On this basis one would expect changes in the sub-microscopic complexes in the body of the cell during its stimulation to be more readily discoverable. And in fact a characteristic change in the physico-chemical state of the protoplasm in stimulated nerve cells has been noted by various authors. Such were the observations of Lavrentiev and Fedorov [15] on surviving ganglionic cells of a frog's heart, where the appearance of protoplasmic granularity was shown on stimulation of the vagus nerve.

Vital staining of nerve cells, subsequent extraction of the stain and its colorometric measurement showed a comparative accumulation of stain in the stimulated nerve cells [16], which the author linked with heightened granule formation. The appearance of granulation in the neuroplasm of the nerve cells of a frog's heart was confirmed in subsequent works, both on stimulation of the vagus nerve and on the action of chemical irritants [19]. Simultaneously, on stimulation, changes were observed in the physico-chemical state of the nucleus and synapses of the nerve cells, and likewise a sharp outlining of the synapses and nucleus, connected with the phenomenon of gelatinization of the cytoplasm of these formations.

Photoelectric registration of the light passing through the visceral ganglion of an *Anodonta* showed an increased absorption by the ganglion on stimulation of the connective commissure [13]. This phenomenon would seem to be connected with change in the dispersed state of the colloid particles.

The material indicated above gives some idea of the physico-chemical and structural state of nerve cells under stimulation. The changes take place mainly in the protoplasm, they are linked with changes in the dispersed state of the colloids of the nerve cell, and with the appearance of granularity, the gelatinization of the protoplasm and a heightening of its tinctorial capacity.

The difficulty of discovering structural and chemical changes increases as one passes on to other parts of the neurone. This may be explained by metabolic processes taking place with greater intensity in the nerve cell than in the dendrites and the axon.

The data of histochemistry and of electron and ultra-violet microscopy show different concentrations of active substances and of sub-microscopic complexes in the body of the nerve cell and in its processes. The enzymatic activity in nerve cells is considerably higher than that in dendrites and axons [20], and in the former is concentrated most of the ribonucleic acid and of some of the aromatic amino acids [21], and most of the mitochondria, lipochondria, microsomes and other structural complexes [22].

Until recently there was practically no direct factual evidence of structural changes in the nerve conductor during transmission of stimulation. This is why the question of the distribution of the nervous impulse has up to now been answered by the purely electrical hypothesis that the transmission of stimuli takes place via an action current flowing in the form of a wave of locally circulating electric currents. The low susceptibility to fatigue of the nerve conductor seemed to bear out this purely physical hypothesis. But the nature of the mechanism whereby stimuli are transmitted is not fully explained by electrical processes or by alteration of the electrical parameters of the nerve conductor. This hypothesis does not explain the facts of the sharply-expressed dependence of speed of transmission on temperature, or the output of heat during transmission of the nervous impulse, and does not enable us to connect the speed of distribution with the basic physico-chemical parameters characteristic of the conductive tissue: the ionic diffusion coefficient, the ionic concentration and the density of the tissue. Nor does it explain the fact of non-decremental transmission of stimuli, or the connexion between transmission and nerve metabolism. The place of the neuroplasm and medullary sheath in the nerve function remains uncertain if one starts from the membrane theory now widely used to explain the phenomena of nerve polarization and the origin of biopotentials.

Attempts to discover ultra-structural changes of the axon during the spread of excitation remained fruitless for a long time. As a result, study of the medullary nerve first took the path of investigating the static electrotonic states in the field of the anode and cathode of a polarizing current applied to the nerve. The opposite electrotonic changes in excitability observed in the fields of the cathode and anode permitted the assumption of corresponding physico-chemical states of the nerve substratum in these fields. It was supposed that the accumulation of polarization changes in the nerve might also assist the accumulation of chemical and structural changes. Kühne [23] long ago found that polarized muscle swelled on the cathode, while Verworn [24] and others described the anode contraction of the protoplasm of protozoa. Munk [25] and Bethe [26] found different susceptibility to staining and opposite changes in nerve diameter in the polar fields of a polarizing current. These studies were developed in subsequent works [27–33]. Investigations of the mechanism of action of cathode and anode on nerve was accompanied by investigations of the interaction of polarizing currents with the antagonistically acting ions—potassium and calcium. But the changes in staining and in nerve diameter in the polar fields, observed in these experiments, were only observed when a comparatively strong current acted on the nerve for a considerable time (tens of minutes or hours), and the changes were accompanied by an irreversible loss of excitability. They could thus be caused by damage, not by normal activity. Besides swelling of the nerve on the cathode and contraction on the anode, changes in optical properties were also found —decrease in light scattering near the cathode and an increase near the anode [34–36].

Similar works have been repeated right up to the present time, on both living and fixed nerves. On prolonged (45–60 min) polarization of frog nerve, for example, and after subsequent fixing and study of transverse sections of the nerve, Pravdich-Neiminskii [37] demonstrated swelling of the nerve fibres on the cathode and contraction on the anode, and explained these changes in terms of redistribution of water and discharge of ammonia.

These experiments did not succeed in showing parallelism in the development of functional and morphological processes, since the electrotonic changes of excitability in the nerve arose very rapidly, while the swelling and the change in staining power appeared considerably later. Attempts were made to transfer the local polarization changes to fit the case of a stimulus being transmitted along the nerve, and to connect anode processes with the state of inhibition and cathode processes with that of excitation [35]. This speculative conclusion, however, did not stand up to criticism and lacked direct experimental confirmation. Meanwhile, attempts to find early structural changes corresponding to functional alterations met with failure. An effort, for instance, to find changes in birefringence on applying stimulation to a single giant axon was unsuccessful [38].

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Among the first successful works on medullary nerve is a study of the viscosity and related properties of the sciatic nerve of the frog [11].

The conditions of the experiment made possible the continuous registration of the viscosity of the living nerve, owing to its being forcibly twisted in opposite directions. As the result of a tetanizing electric excitation, an increase in the nerve's viscosity slowly developed, which originated either at the time of excitation, or subsequently after a latent period lasting up to 40 sec. The same authors [39] in a later work found a more rapid change, occurring at the actual moment of excitation, in the tension of the sciatic nerve. During electrical and mechanical excitation of the giant axon of the squid (*Loligo pealii*), a decrease in the speed of outflow of axoplasm from the severed end of the nerve was observed [40] which was explained by the author as due to gelatinization of the neuroplasm and an increase in its viscosity.

Structural changes in an irritated nerve were later shown by optical methods. Photoelectric registration of the light scattered by a nerve made possible the observation of slowly built-up changes in the light-scattering capacity of the non-medullary nerves of the crab and cuttlefish upon irritation by a series of impulses [41, 42], and a connexion was found between these changes and the osmotic pressure of the surrounding medium. Later it was found [43] that the light scattered by the nerve provides a very sensitive test for the very slight changes in the thickness of the nerve brought on by change in the osmotic pressure of the surrounding medium and by electrical irritation. Irritation of the single giant axon of the cuttlefish for 1 min by a current of frequency 200 c/s evoked a swelling of the fibre by 0·1–0·2μ. It is characteristic here that the swelling was maintained for several seconds after cessation of the irritation.

Then came success in showing optical changes in medullary nerve. By means of photoelectric registration, changes in the transparency of a nerve during irritation were observed on the fine nerve fibre innervating the sartorius muscle of a frog [12]. The changes in transparency began at the time of irritation and continued to build up for some time after its cessation. They resulted from a kind of integration of a

number of irritating impulses. These data were soon confirmed by American workers [44], on the sciatic nerve of the frog as well. Here even slower changes in the light-scattering capacity of the nerve were shown. They began several seconds after the start of irritation and were observed for some time after its cessation.

Physico-chemical processes in irritated non-medullary nerves were also demonstrated by staining *in vivo*. In a crab nerve a heightened sorption of methylene blue is observed upon stimulation [45]. A heightening of the sorption level of stain has also been demonstrated on *Anodonta* nerve during stimulation. Furthermore, a decrease in colouring was observed which corresponded to the decrease in amplitude of the excitation waves spreading along the nerve. But work on the stain sorption of irritated frog nerve did not show very definite changes when compared with the control [47]. It was assumed that the medullary sheath prevents this factor coming out clearly. A study was therefore made subsequently of the branchings and endings of the sciatic nerve of the frog, which are without medullary sheath, and a heightening of staining capacity was found as a result of irritation [48].

For an understanding of the nature of optical phenomena in the nerve it is very important to consider them in conjunction with the electrical processes. The first step in this direction is the registration of the structural changes making themselves apparent upon a single stimulus. All the works mentioned above succeeded in noting only the effect of a series of irritant impulses. A response to a single stimulus was revealed in the connective nerve commissure of *Anodonta*, by photoelectric registration of the light passing through the nerve [13]. A single stimulus, applied to the end of the nerve commissure, evoked a wave of increased opacity spreading to the visceral ganglion and the post-ganglionic branches. After a single stimulus a late effect was observed in the form of rhythmical variations in transparency. The two-phase optical changes appearing as after-effects are very similar in character and timing to the prolonged two-phase electrical after-action observed in the connective nerve of *Anodonta* and in other non-medullary nerves [49, 50]. This provides a foundation for the hypothesis that structural and electrical processes in stimulated nervous tissue are in some way interconnected.

Light-scattering changes were later found when a single stimulus was applied to the extended nerve of a crab. But no direct comparison in time of optical and electrical changes was made owing to inherent difficulties of method. When the photocurrent is amplified to the strength required for the registration of slight changes in the intensity of the light scattered or transmitted by a nerve, the level of "noisy" interference becomes high. The presence of interference makes it impossible to note exactly the beginning of the optical effect, which starts gently and gradually. Such a comparison in time was made subsequently on a medullary nerve, using a more sensitive optical method not requiring amplification. The photoelectrical method was unable to reveal any changes in medullary nerve upon passage along it of a single impulse. The comparatively slow optical changes observed upon irritation by a series of impulses, although reflecting the activity of the nerve, could not be directly compared with action currents, and it was therefore hard to evaluate their connexion with the spread of excitation along the nerve.

In order to interpret these facts in their connexion with the spread of excitation in medullary nerves it was essential to study the time factor for the structural changes

and compare this with the biopotentials. This proved to be an exceptionally difficult task, owing to the need for a very sensitive optical method, since changes observed in the light-scattering and transparency of irritated nerve had been apparent only because of the build-up of such changes under the action of a series of impulses.

Numerous attempts to discover the effect of a single stimulus have succeeded recently thanks to the use of the micro-interference method [51, 52]. The interference of reflected light on the surface of the sciatic nerve of a frog was studied, upon a single stimulation. Observations were made of the deflexion of the interference bands, which was evidence of a displacement of the surface of the nerve, this being restored to its previous position after the cessation of the stimulus. This shift in the nerve's surface reflected a compressional wave, passing along the nerve as excitation spread. The extent of the expansion and contraction observed was not more than 0·03–0·04μ; in relation to the diameter of the whole nerve trunk this was about 0·03 per cent. In control experiments on anaesthetized nerves, incapable of excitation, and also with application of a subliminal irritant to a normal nerve, surface displacement was not present. Later, successful registration was made of the displacement of the interference bands of the nerve with a longer time-interval and simultaneously with the biopotentials. This comparison showed that the single wave of expansion and contraction in the nerve begins simultaneously with the spike, but begins less sharply than the jump of the potential and is considerably slower in its development taking in the period of the low-voltage follow-on potential [53]. The duration of the volumetric-resilient displacement in the nerve is about 0·05–0·1 sec, coinciding in time with the "metabolic tail" of the biopotential.

The question arises, are changes in volume as universal a characteristic of the wave of excitation as are electrical changes? The fact that they were observed when both medullary and non-medullary nerves were stimulated allows us to assume them to be a common property of all types of nerve. From this point of view especial interest attaches to the changes in volume in contracting frog muscle [54]. They take place with considerably greater speed than the mechanical processes of contraction, coming close to the currents of action in this respect. Their connexion with some links in biochemical metabolism during muscle contraction has been established.

The result of a large number of studies has been to create the opinion that the process of excitation is always accompanied by action currents, but that, as far as optical changes are concerned, in a normally conducting nerve it is often impossible to observe these.

This may, however, be the result of our not knowing all the conditions necessary for discovering the structural changes taking place during a nerve's activity. One factor may be the insufficient sensitivity of optical methods, or the masking of effects by the physical and chemical conditions of the surrounding medium. Changes in a nerve's properties of resilience and light-scattering, for instance, are brought out much better when suitable conditions of stretching of the nerve are observed, determining the tension and orientation of the anisotropic sub-microscopic particles. An electrical reaction of the nerve is much easier to discover, and is not masked by such factors as diffusion of light by the medullary sheath, the formation of folds, or the loss of orientation by the anisotropic particles.

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In discovering optical reactions of a nerve, vital importance attaches to preserving the connexion between the nerve cell and the axon under observation. A nerve shortens after being cut across, which indicates the existence of a stretching force that acts under normal conditions and may, perhaps, determine the orientation of the structural elements.

The considerable difference between nerve cell and axon in the concentration of active chemical substances may be a factor creating a gradient of hydrostatic pressure within the nerve. By cutting across the nerve process we destroy the action of these factors and reduce the extent of structural effects. Disorientation of structure in a cut nerve can be compensated to a certain extent by stretching, which raises the internal hydrostatic pressure and orientates the particles. Observance of this condition gives a fairer demonstration of change in light-scattering during transmission of excitation in non-medullary nerve and of the volume-resilience wave in medullary nerve [52].

Lastly, direct experiment has shown that changes in light scattering are demonstrated with greater reproducibility in the nerve commissure of *Anodonta* when connexion with the nerve ganglia is preserved, whereas severance evokes considerable changes in the effect, and often its disappearance.

Taking all the above into account, we feel that structural and chemical changes are a no less constant expression of excitation than biopotentials. The comparatively protracted nature in time of resilience and structure effects is far from being an indication of their secondary character, and only shows that excitation is not confined to the spike phase, but takes in a period of time of the order of 0·1 sec, thus approaching the time taken by a single muscle contraction. Therefore, peak and metabolic potentials should not be separated as physical and chemical phenomena. There is an integral process of excitation, including the formation of the impulse and of the active chemical substances. From this point of view, excitation does not end with the spike, but is a more lengthy process. Ultra-structural and volumetric changes, beginning with the spike and taking in the metabolic potential, make one whole of the physical and chemical sides of excitation.

In connexion with the complex, multi-phase structure of medullary nerves, the question arises of the localization of changes of the optical, volumetric and viscous properties. There is comparatively little direct evidence that the neuroplasm of nerve processes and nerve cells changes its physico-chemical state during excitation, and what observations there have been are only qualitative. They include the observations mentioned earlier, by Lavrentiev and Fedorov [15], and those of Flaig [40], who found that irritation increased the viscosity of the axoplasm flowing from the severed end of the giant axon of a squid. One may also bring under this head the experiments performed by Solomon and Tobias [55], introducing light into the giant axon by illuminating a quartz needle inserted down the length of the nerve. In front of the needle, in the undamaged neuroplasm, a cone of diffused light appears, showing the opacity of the neuroplasm. As the needle moves along within the fibre, Tyndale's cone disappears in the field of excitation, i.e. the scattering of light decreases, and Brownian movement of the large particles becomes noticeable.

The changes in optical density observed during nervous activity in non-medullary nerves and separate fibres would seem to be connected, not only with re-distribution

of ions and water, as Hill thinks [42, 43], but also with a reversible gelatinization of the axoplasm. Confirmation of this is provided by those experiments in which the gelatinization of agar is shown to be accompanied by heightened scattering of light [56], as a result of which the clear solution becomes opalescent.

The part played by the medullary sheath in excitation remains uncertain. But the localization of enzyme systems such as acetylcholine-esterase [57], cocarboxylase [58], and adenosine-triphosphatase [59], and also of some proteins (tryptophane [60]), on the surface of non-medullary axons and in the medullary sheath of medullary nerves, is evidence of the sheath being concerned in the execution of metabolic processes in the nerve.

The facts available on metabolism of nucleic acids, enzymes and nucleoproteids in the process of nervous activity indicate a connexion between structural changes and the physico-chemical state of proteins. The study of protein transformations in the process of nervous excitation is difficult, demanding extremely sensitive methods of dynamic analysis. One such method is ultra-violet microspectrophotometry, which is based on the fact that a large number of biologically important protein substances display characteristic absorption maxima in the ultra-violet field of the spectrum.

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Change in absorption of ultra-violet radiation on the living nerve fibre can help to elucidate the chemical organization of the nerve, the way in which this alters during the process of excitation, and also the significance of some protein substances for nervous excitation. But the use of ultra-violet radiation introduces additional difficulties, connected with the high sensitivity of the proteins under study to the action of such radiation, which denatures them. The first studies of this kind [61–63], on a whole sciatic nerve (of a frog) in Ringer's solution, showed a gradual increase in chronaxie, terminating in loss of excitability and disappearance of currents of action, under the action of ultra-violet rays. Such an action is exerted only by ultra-violet radiation with a wavelength of less than 310 mμ. Subsequently, studies were made of a single medullary fibre [64, 65] under isolated X-irradiation of the Ranvier nodes and the internodal sectors protected by the medullary sheath. This showed the nodes to have a considerably greater sensitivity to radiation than the internodal sectors. A dependence between sensitivity and wavelength was noted, and wavelengths of 280 and 265 mμ found to be the most effective.

Muralt and Stämpfli [66] have made a more detailed study of the photochemical action of ultra-violet radiation on a single medullary fibre, and its dependence upon wavelength and intensity of radiation. They confirm the data on the difference in reaction and sensitivity between the nodes and the internodal sectors. Upon irradiation of the node alone, the threshold of excitation immediately starts to rise, while irradiation of the myelin-protected area evokes an equally prompt reduction in the threshold, which is replaced after a short time by a steady rise continuing until excitability disappears completely. The amount of radiation needed to produce disappearance of excitability in the area of the nodes of Ranvier is only one-fiftieth of that required in the case of the internodal sectors. Furthermore, irradiation of the nodes showed three maximal points of photochemical activity in the short-wave ultra-violet region (297, 280 and 265 mμ), whereas the photo-chemical activity curve for the myelin-protected parts does not exhibit these peaks.

In recent works from this laboratory [67, 68] studies along these lines are helped by a new method, stroboscopic photochemistry, which makes it possible to set the photochemical action of ultra-violet radiation against the nervous impulse. Short rhythmical bursts of ultra-violet are synchronized with even shorter irritant impulses of the same frequency. The authors study changes in the thresholds of a single medullary fibre in relation to three different patterns formed by the burst of light and the irritant impulse: (1) coincidence of the peak of the light-burst with that of the irritant impulse; (2) coincidence of the peak of the light-burst with the end of the action current; (3) the peak of the light-burst precedes the action current. The authors demonstrated that the photochemical action of ultra-violet rays is strongest when the burst of light and the action current coincide in time. The excitability of the nerve declines most rapidly in this case. If the light impulse comes at the end of the irritant impulse, or precedes it, the photochemical action of the radiation is weaker, and the threshold of excitation goes up slowly. The experiments mentioned also show that the spectral region at $\lambda = 265$ mμ has the strongest photochemical action, the 280 mμ region being less effective. The great increase in photochemical sensitivity in the node at the moment of excitation indicates that during the process of excitation metabolic processes in the proteins of the axoplasm are under way, as well as ion displacement. Direct data on the content of some proteins and nucleic acids in medullary and non-medullary nerve have been obtained by Lüthy [60, 69]. In a medullary fibre from a frog's nerve at rest, the absorption of ultra-violet radiation is characterized by three peaks, at wavelengths 248–250, 265 and 280 mμ.

The absorption peak at 248–250 mμ is connected with the thiamine content of the nerve, or that of its active form, cocarboxylase; the 265 mμ peak corresponds to the nucleic acid content; and, lastly, peaks at 279 and 280 mμ are connected with the content of the aromatic amino acids tyrosine and tryptophane.

For the non-medullary giant fibre of the earthworm, the characteristic absorption curve has one main peak in the 260 mμ region and a minor one at 280 mμ. A two-peak absorption curve is also characteristic of the olfactory nerve of the pike.

When absorption in the 265 mμ region during irritation was measured, it was found to rise by 3–5 per cent. Does this effect correspond to a real rise in nucleic acid content in the nerve? If one takes in account changes in light-scattering and in the diameter of the nerve occurring on irritation, the decrease in intensity of the ultra-violet light passing through the nerve may be ascribed to these factors as well as absorption. But in some experiments Lüthy succeeded in showing a rise in absorption in the 265mμ region not in the nerve fibre itself, but alongside its severed end, from which some active substances flow. Disintegration of some proteins during excitation has been observed in rat nerve [70]. After irritation of the nerve for a few seconds the ultra-violet absorption of alkaline nerve extract (pH 12) increases at certain definite wavelengths. These changes are evoked by brief irritation, they are reversible, and could consequently be discovered only immediately after irritation.

There is, thus, confirmation for the hypothesis that during excitation liberation and accumulation of certain substances of a protein nature takes place. This does not run counter to other known facts.

The importance of chemical substances in the peripheral nervous system has been closely studied in connexion with the part played by mediators in the transmission of

excitation from the nerve endings to the effector organs, and in interneural formations. It later appeared that active substances were discharged not only by the nerve endings, but also along the whole length of the nerve. On irritation of a nerve, acetylcholine and thiamine are discharged from its severed end into an irrigating fluid [71, 72]. The secretory function of the nerve is also expressed in the discharge of drops of liquid from the Lentherman's notches, as is demonstrated on irritation of medullary nerve [73, 74].

The secretory function is linked with the ability of some substances to travel along the nerve. A study of this phenomenon was carried out by Weiss [75], who showed that, after ligaturing, the central part of the nerve swells, pointing to a continual movement of the nerve substance in a peripheral direction. The movement of these substances is a very slow process, according to Weiss's calculations taking place at a speed of 1 mm per 24 hr, which corresponds to the speed of regenerative processes. On severance of a fibre a difference between the central and peripheral portions is very quickly apparent. It begins with a bulging out of the axoplasm of the central section, under the influence of intracellular pressure. The axoplasm of medullary and non-medullary nerves is a viscous liquid, able to flow along the fibre. However, the liquid is characterized by a regular organization of molecules, proved by the existence of birefringence and nerve fibrils. It is possible that this regularity is due to the influence of the internal pressure of the fibre.

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Outflow of the axoplasm can also be observed in an isolated section of nerve. This phenomenon has been studied quantitatively in single medullary fibres [76, 77]. It has been established that the outflowing spherical drop of axoplasm is covered by a fine layer of myelin spreading out over it. The volume of the outflowing axoplasm depends on the diameter of the nerve and is proportional to the square root of time.

This property of the axoplasm, of flowing along the nerve, undoubtedly plays an essential part in the passage away from the nerve cell not only of water and salts, but also of various proteo-lipoid complexes. This current may transport nucleoproteins [9, 10], neuro-secretory substances [78] and other substances organized into submicroscopic structures [22].

Exchange between the nerve and its environment has been studied in many works from the standpoint of the membrane theory. But in these cases the matter under discussion was confined to the passage of some ions and of water in a direction across the nerve, and their significance for the generation of the spike.

We feel that the migration of substances along the nerve is of essential importance not only for the growth and regeneration of the nerve, but also in connexion with the transmission of excitation along it.

Although the movement of the axoplasm along the nerve is a slow process, exercise of the active transmitting function of the nerve should speed up the flow of these substances. This can be forecast theoretically on the grounds of the wave-like character of the excitation process, which spreads with a constant speed. Reckoning with this wave-like nature of the transmission of nervous excitation, one can visualize how in the nerve the pre-excited state is separate from the state excited by the front of the wave. This leads to changes in density or in volume of the tissue and to the start of a flow of substance. The volumetric-resilient variations in the nerve which

accompany the nervous impulse [51–53] must in their turn evoke some small displacement of substance in the direction of movement of the impulse.

The mechanism of migration of substances in the axoplasm is not determined by changes in resilience of the nerve alone, but also by the presence of a constantly acting disparity in the concentration of active substances. A sharp difference in the quantities of protein substances and enzymes contained in the body of the nerve cell and in the axon can be a continually acting cause of, and a necessary prior condition for, an equalization of the concentrations of these substances by means of ion-diffusion processes.

The flow of liquid in the nerve can be proved by direct optical observation [79]. Using the Tolpler shadow method, extremely small changes in the refractive index of the medium can be distinguished, and the stream of liquid flowing from the end of the nerve can be clearly seen and photographed. This stream of liquid is evidence of considerable hydrostatic pressure within the nerve. The speed of outflow is determined by the difference between the refractive indices of this stream and of the surrounding Ringer's solution. Successful observation has been made in this way of the increase in the stream's speed during irritation.

Recently published works [80] report successful experimental demonstration of the migration of solid substances in motor nerves. During electrical irritation of the nerve a rapid movement of solid substances along the nerve towards the periphery takes place. The transfer of solids during irritation takes place so quickly that a temporary accumulation of them is formed in the distal part of the nerve; this is shown by the increase in dry weight in this part. A rapid transfer of solids along the nerve also takes place under reflector irritation of the motor nerve. A slower transfer of solids in motor nerves takes place in a centrifugal direction and without the passage of a nervous impulse [81].

All these facts taken together direct attention to the phenomenon of the transportation of liquid axoplasm and solid substances along the nerve. It is difficult to overestimate the possible significance of this process for the trophic function of the nervous system. The well-known fact that atrophy of the muscle develops after severance of the motor nerve is connected with a considerable change in muscular metabolism. It is possible that substances vital for the maintenance of normal metabolism are transferred towards the muscle along the motor fibres from the nerve cell, stimulated by the excitation spreading along the nerve.

What is the mechanism of transfer of substances along the nerve? Are they transferred along with the wave of excitation from the nerve cell in an unchanged state, or does the wave of excitation, as it spreads, evoke a chemical and structural reorganization of their complex associations and the freeing of certain substances which then spread by diffusion or other means? Both ways seem possible, but the question calls for direct experimental study.

The data adduced bear witness to the interweaving of chemical, physical and structural phenomena in the transmission of excitation along the nerve. We visualize the passage of the nervous impulse as a complex physico-chemical process. The rapid, leading part of this process, reflected in the spike, is supported by slower processes which aid the convectional transfer of matter along the nerve. In this case there is no need to consider the low-voltage potential as a rehabilitatory phase. The

factors which have been kept so far apart, looked at from the standpoints of physics and chemistry respectively—the spike on the one hand and the metabolic action on the other—can be considered as a single process. From this point of view excitation does not end with the spike, but is a more prolonged process.

Translated by R. KISCH

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STUDY OF THE SIZE AND SHAPE OF THE IMPEDANCE SPIKE IN THE FROG NERVE AT VARIOUS INTERELECTRODE DISTANCES*

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(*Received* 7 *January* 1957)

IN animal physiology, since the end of the last century, when the problem of excitation has been discussed the concept of membrane permeability has been used. In time these ideas have undergone careful testing and have been further developed on the basis of a large amount of electrophysiological work.

* Biofizika **2:** 5, 602–613, 1957 [Reprint Order No. BIO 79].

At the present time two circumstances support the membrane concept: the existence of a potential difference between the internal and external media of the cell [1] and the cable-like structure of a nerve which has been established by study of its passive electrical properties [2, 3]. Also, a great number of factors are concretely explained on the basis of the membrane theory of excitation and this indicates its utility.

However, for the membrane hypothesis in myelinated nerve fibres, more direct facts, indicating that the surface structures of the axoplasm (the membrane) form the active substrate on excitation, are necessary.

At the same time another point of view, of which the basic idea was also formulated at the end of the last century, has become widespread in the physiological literature. The active substrate on excitation is considered to be the whole mass of the axon protoplasm ("phase" theory of excitation [4, 5, 6, 7]). A special role of the surface structures of the protoplasm (membrane) is denied.

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Thus, two different points of view exist: one considers only the surface structures of the axoplasm to be active on conduction of excitation (membrane theory), while the other considers all the axoplasm (phase theory). It seems to us that the possibility of recording an impedance spike on the nerve trunk of vertebrates creates conditions suitable for determining which substrate is the vehicle of conduction of excitation in myelinated fibres. Our work is concerned with the analysis of this question.

THEORETICAL PREREQUISITES TO THE STUDY

The drop in impedance during the passage of waves of excitation along a nerve is explained in different ways by the membrane and phase theories. From the point of view of the first, the decrease in impedance is explained by an abrupt increase in the electrical conductivity of the membrane, connected with an increase in permeability for certain ions [9, 10]; from the point of view of the second, the impedance change is due to an increase in the electrical conductivity of the axoplasm itself, connected with dissociation of its protein-electrolyte complex [11].

Thus, there would be interest in the type of experiment which would determine, in a well-defined way, which substrate is concerned in the change in electrical conductivity on excitation. The possibility of finding such an experiment is connected with the features of the flow of electrical current through myelinated nerves. As a starting point for the analysis of the electrical current flow through nerves, we take the experimental curve, obtained by a number of authors, of the change of resistance of a nerve with distance between electrodes (s)—the curve OA (Fig. 1).* The nonlinearity of the curve OA for small s=8–10 mm points to the peculiar heterogeneity of the nerve conductor. This fact, and also the morphology of myelinated nerves, logically leads to the consideration of three basic elements which make up the total resistance of the nerve: (1) the extracellular liquid bathing the nerve fibres; (2) the axoplasm; (3) the sheathing structure of each axon which separates the first two elements.

The last element has, basically because of the myelin sheath, a considerably greater specific resistance than the first two; therefore the current through it passes

* By the distance s, here and subsequently, is implied the distance between electrodes, through which the testing current is passed for determining the resistance of the nerve.

only in the radial direction, with respect to the nerve axis. Similar ideas on the electrical structure of the nerve fibre have recently found experimental support from direct measurements on single myelinated fibres [12].

Having carried out a mathematical analysis of the distribution of current in "cable-like" structures, Rushton [13] established the following law of change of resistance with increasing distance*:

$$T = \frac{\lambda r^2}{r+\sigma}(1-e^{-s/\lambda}) + \frac{sr\sigma}{r+\sigma}\dagger \tag{1}$$

where T is the resistance of the nerve between the electrodes; s is the interelectrode distance; σ is the resistance of the axoplasm per unit length; r is the resistance of the extracellular fluid per unit length; R is the resistance of the sheath per unit length; λ is the space constant determined by the following equation:

$$\lambda = \sqrt{\frac{R}{r+\sigma}} \tag{2}$$

It is easy to see that this formula analytically describes a function which corresponds to the experimentally obtained curve shown in Fig. 1 (OA). The first term on the right-hand side of the equation corresponds to the change in the sheath resistance with increasing s and the second to the change in the resistance of the axoplasm and extracellular fluid. The sheath component of the resistance grows exponentially and with further increase of s ($s>8$–10 mm with $\lambda=2$–3 mm) becomes

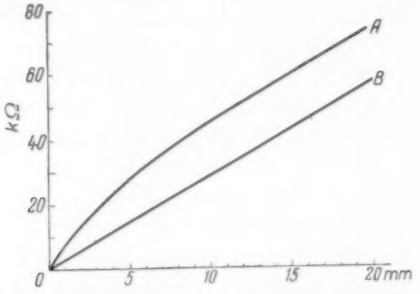

FIG. 1. Curve OA corresponds to the change in resistance of the nerve with increasing distance between the impedance electrodes. Ordinate—the resistance of the interelectrode portion of the nerve in kΩ; abscissa—the interelectrode distance in millimetres. The figure is taken from Rushton [13].

practically constant; in Fig. 1 it corresponds to the vertical difference between the lines OA and OB. The component of the resistance due to the axoplasm and the extracellular fluid is proportional to s and corresponds to the straight line OB in Fig. 1.

Thus, the change in resistance of the nerve with increasing interelectrode distance is determined by the following considerations. For small s most of the current flows through the extracellular fluid. With increase of s the resistance of the extracellular

* It must be noted that, in order to simplify the mathematical analysis, Rushton did not take into consideration the nodes of Ranvier; however, he pointed out that if they were taken into account the result would be the same.

† Rushton's symbols are used.

fluid increases proportionally and there is an increase in that part of the current penetrating through the sheath of the nerve fibres which correspondingly increases the share of the axoplasm in the longitudinal conduction of electric current, and so causes a fall in the gradient of the curve OA, i.e. a slowing down of the growth of the resistance with increasing s. On further increase of s, the part of the current passing through the sheath into the axoplasm becomes practically constant and the function T becomes linear.

From formula (1) one can obtain two functions (Q_1 and Q_2) each determining the relative change of the contribution to the total resistance of the nerve by the given component with change of s. We must, however, take into account that the contact resistance between nerve and electrodes, a constant K, is always added to the total resistance of the nerve (T) under the experimental conditions. From this we have, for component I of the resistance (the sheath):

$$Q_1 = \frac{\text{component I}}{T+K} = \frac{\dfrac{\lambda r^2}{r+\sigma}(1-e^{-s/\lambda})}{\dfrac{\lambda r^2}{r+\sigma}(1-e^{-s/\lambda}) + \dfrac{sr\sigma}{r+\sigma} + K} = \frac{1}{1+\dfrac{sr\sigma + K(r+\sigma)}{\lambda r^2(1-e^{-s/\lambda})}}; \quad (3)$$

for component II of the resistance, axoplasm and extracellular fluid, we correspondingly have:

$$Q_2 = \frac{\text{component II}}{T+K} = \frac{sr\sigma/(r+\sigma)}{\dfrac{\lambda r^2}{r+\sigma}(1-e^{-s/\lambda}) + \dfrac{sr\sigma}{r+\sigma} + K}$$

$$= \frac{sr\sigma}{\lambda r^2(1-e^{-s/\lambda}) + sr\sigma + K(r+\sigma)}. \quad (4)$$

Q_1 and Q_2 are represented graphically (for $K=0$) by curves AB and CD in Fig. 2.

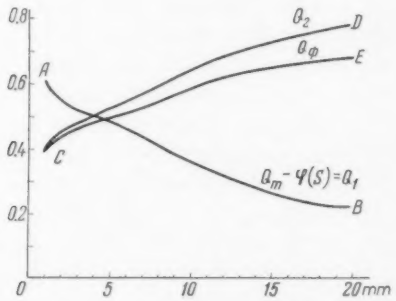

FIG. 2. Curve AB corresponds to relative change in resistance of the sheath component with increasing s, and curve CD to the component, due to the axoplasm and the extracellular fluid, of the total resistance of the nerve. Ordinate—the fraction of the total resistance of that part of the nerve enclosed between the electrodes.

The magnitude of the effect of a change in electrical conductivity of the system, due to any process, will always be proportional to that part of the resistance, in the total resistance of the system, which determines the effect.

With regard to the ideas of the membrane theory, the effect of a change in electrical conductivity on excitation is determined by structures whose resistance is included in the sheath component of the nerve resistance. From the point of view of the phase theory this effect should be determined by elements whose resistance is included in the second component of the total nerve resistance (i.e. by the axoplasm).

Thus, our functions Q_1 and Q_2 should characterize the relative change in amplitude of the impedance spike with increase of interelectrode distance for the membrane (Q_1) and the phase (Q_2) theories, respectively.

For complete characterization of the change in amplitude of the impedance spike with increasing interelectrode distance, it is necessary to consider the influence on the recorded amplitude of the shape and length of the spatial distribution of the electrical conductivity changes along the nerve during excitation (dotted line in Fig. 3).* The distance of the dotted curve from the line OP gives, at each point, the

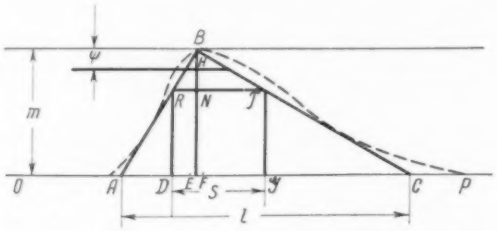

FIG. 3. Diagram explaining the decrease of the impedance spike on increasing the interelectrode distance which is connected with the spatial distribution of the electrical conductivity changes, on excitation, along the nerve. The value of 1, necessary for the passage of ψ for any s is 35 mm (explanation in text).

relative magnitude of the conductivity changes in the corresponding part of the nerve (line OP). In order to simplify the calculations we will consider the shape of the impedance spike to be the inscribed triangle ABC (Fig. 3). If the interelectrode distance is small ($s=1$ mm) then the part of the nerve included between the electrodes, E and F, will be practically wholly subject to the greatest changes in electrical conductivity at the instant shown in the figure, and thus the average size of the conductivity changes in the part EF of the nerve corresponds to the height m of the triangle. If the electrodes are moved apart a distance s (electrodes D and G) then, even at the most favourable instant of passage of the wave of excitation, indicated in Fig. 3, the average size of the conductivity changes in the part DG of the nerve will be smaller. If we assume that all parts of the nerve in the interelectrode region are equivalent with respect to recording the impedance changes then, from the diagram in Fig. 3, it is easy to see that in this case the average size of the conductivity changes will correspond to $m-$BH, where H is the point of intersection of the vertical of ΔABC and the median line of ΔRBT. Since the maximum amplitudes of the recorded impedance spikes are determined by the average magnitudes of the conductivity changes in the section between the electrodes at the most favourable instant of passage

* The spatial spread of the impedance spike has been found from the time change in electrical conductivity, recorded by us during excitation [8]. Knowing the speed of the excitation wave and the time of passage of impedance changes past one point, one can speak of a wavelength equal to 40–50 mm.

of the wave of excitation, then the changes of these magnitudes will be proportional to each other. Knowing the length BH=ψ for any s, we can always calculate this factor. From Δs ABC and RBT we have $m/l=2\psi/s$; hence

$$\psi = \frac{ms}{2l}. \tag{5}$$

From the point of view of the phase theory all parts of the nerve in the interelectrode region are equivalent as regards recording impedance changes, hence, for this theory, the derived relation is valid. Since in this case m corresponds to Q_2 with $s=1$ then for ψ, when comparing the amplitudes of impedance spikes, we have:

$$\psi = \frac{r\sigma}{\lambda r^2(1-e^{-s/\lambda})+r\sigma+K(r+\sigma)} \cdot \frac{s}{2l}. \tag{6}$$

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Since this process for increasing s leads to decreasing amplitude of the impedance spikes, then for Q_ϕ (the function Q_ϕ is the comparative change of the impedance spike with increase of s, for the phase theory) we shall have:

$$Q_\phi = Q_2-\psi = \frac{sr\sigma}{\lambda r^2(1-e^{-s/\lambda})+sr\sigma+K(r+\sigma)} - \frac{r\sigma}{\lambda r^2(1-e^{-s/\lambda})+r\sigma+K(r+\sigma)} \cdot \frac{s}{2l}. \tag{7}$$

The graph of this function corresponds to the curve CE in Fig. 2.* As is clear from the graph, this function increases for the first 20 mm. This function has a maximum in the region of $s=30$ mm.

According to the membrane theory, the parts of the nerve in the interelectrode distance are not equivalent as regards recording the impedance spike. This is due to a non-uniform distribution between the electrodes of the density of the current crossing the membrane [13, 14]. The greatest current density is in the neighbourhood of the electrodes, and it then falls quasi-exponentially, equalling zero halfway between the electrodes. In this case the mathematical characterization of the above-indicated process is more complex and expression (6) does not correspond to it. Since the function Q_1 falls with increasing s, the phenomenon of the decrease of the amplitude of the impedance spike—connected with the spatial distribution of the electrical conductivity changes on excitation, along the nerve—cannot change the general tendency of the function Q_1 for any s, as it can for function Q_2. Without considering this case mathematically, let us denote this process of decrease of the amplitude, for the membrane theory, by $\Psi(s)$. Then for Q_m (the function Q_m is the change of impedance spike with distance for the membrane theory) we shall have:

$$Q_m = Q_1-\Psi(s) = \frac{1}{1+[sr\sigma+K(r+\sigma)]/\lambda r^2(1-e^{-s/\lambda})} - \psi(s). \tag{8}$$

From the equation it is clear that the curve Q_m is always lower than the curve Q_1 (curve AB in Fig. 2).

Thus, we have obtained theoretically two different curves for the relative change in amplitude of the impedance spike on excitation as a function of the interelectrode distance. If in fact the amplitude of the impedance spike behaves like the function

* One can satisfy oneself by inspection of Fig. 3 that our assumed simplification of the shape of the impedance spike would lead to an increase in the function ψ for any s; therefore, using the real shape of the impedance changes, we would obtain a curve always lying above CE.

Q_m, equal to $Q_1 - \Psi(s)$ (in Fig. 2 curve AB corresponds to Q_1), then the substrate which determines the electrical conductivity change on excitation is in the surface structures of the nerve fibre (the membrane). But if the amplitude of the impedance spike behaves like the function Q_ϕ (in Fig. 2 curve CE corresponds to Q_ϕ), then the active substrate which determines the change in electrical conductivity is the whole mass of the axoplasm.

It is in connexion with these ideas that our work is devoted to the study of the size and shape of the impedance spike for various interelectrode distances.

METHODS

We used a bridge method of recording the changes on excitation of the electrical conductivity of the nerve. A cathode ray oscillograph was used as a null indicator. We judged the magnitude of the electrical conductivity change by the amount of unbalance, which appeared on the oscillograph screen as a symmetrical impedance spike. A description of the apparatus used has been published [8].

In this work we used the sciatic nerve of the frog (*Rana temporaria*). So as to decrease the shunting effect of the extracellular fluid, the surface of the nerve was carefully dried before putting it in the hermetically-sealed moist chamber. In the chamber were arranged 20 platinum electrodes of diameter 0·3 mm, which served both for stimulating the nerve and for applying the alternating testing current. Such a chamber construction with a large number of electrodes was very convenient for rapidly changing the interelectrode distance; for this it was sufficient to switch the terminals of the corresponding electrodes.

In order to decrease experimental error when measuring the impedance spike, we varied the interelectrode distances on a piece of the sciatic nerve, deprived of branches, from $s=1$ mm to $s=22$ mm.

During all the measurements on the nerve the electrodes in the chamber were not moved. The state of the nerve was determined by its excitability, by the maximum size of the action potentials, and also by the change of electrical conductivity. During an experiment these parameters changed by less than 5 per cent.

The frequency of the alternating current which was applied to the nerve was 35 kc/s. Its voltage at the bridge input was 100–200 mV which, at the frequencies we used, was considerably lower than the stimulation threshold.

The course of an experiment was as follows. First, the maximum strength of stimulation for the given nerve was determined. Then the alternating (testing) current was applied across a piece of nerve of the necessary length enclosed between the electrodes. After balancing the bridge, rectangular pulses of maximum strength, of frequency 50–70c/s and of length 50–100μ sec, were sent through the stimulating electrodes. The impedance spike then observed on the oscillograph screen was either photographed or its magnitude measured on a millimeter scale. Measurements of impedance spike amplitude for each interelectrode distance were carried out a few times.

It should be noted that the sensitivity* of our arrangement changed (decreased) somewhat with increase of the active component of the impedance which occurred

* The sensitivity of the arrangement was measured in millimetres of broadening of the oscillograph beam for a change in the active (or capacitative) component of ± 1 per cent.

on increasing the interelectrode distance. In this case the sensitivity of the apparatus was specially adjusted. In all the experiments of this series, for all interelectrode distances, one and the same sensitivity of the apparatus, 23 mm/$\pm$0·1 per cent, was used. The experiments were carried out at a temperature of 19–20°.

ANALYSIS OF IMPEDANCE SPIKE AMPLITUDE WITH CHANGE IN INTERELECTRODE DISTANCE

Typical curves, for this series of experiments, for the dependence of nerve resistance on interelectrode distance are shown in Fig. 4. As is clear from Fig. 4, the features of these curves fully correspond to the curves obtained by Rushton and later workers [13, 15]. One can notice only one difference between our curves and those

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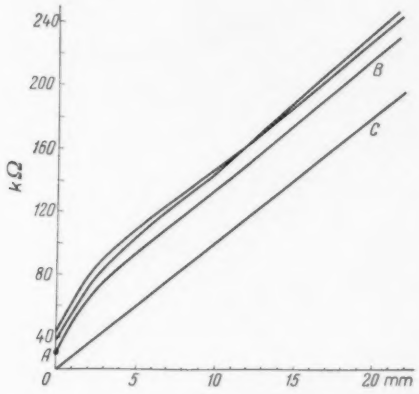

FIG. 4. The presented curves show the change in the nerve resistance with increasing s in our experimental conditions. Ordinate—resistance in kΩ; abscissa—interelectrode distance in millimetres.

of the above authors. It is that in Fig. 4 the function T is already linear at s=4–5 mm. This difference is evidently due to the fact that, by drying the surface of the nerve trunk, we achieved the greatest possible increase in r (the resistance of the extracellular fluid) compatible with the normal functioning of the nerve. As can be seen from formula (2) this, in its turn, leads to a decrease of the space constant λ, whence the function T becomes practically linear for small s.

It is also clear from Fig. 4 that our curves do not pass through the origin of co-ordinates. The intercepts OA, etc., from the origin of co-ordinates to the beginning of the curves of Fig. 4, correspond to the additional contact resistance (K) between the electrodes and the nerve trunk.

As has already been pointed out above, the basic task of our investigation is a comparison of impedance spike amplitudes recorded at various interelectrode distances. In Fig. 5 typical graphs (continuous, disjointed, lines) are presented of the relative change of the impedance spike amplitude with increasing interelectrode

distance. For comparison are presented (dotted) the functions Q_ϕ (curve CD) and $Q_1=Q_m-\Psi(s)$ (curve AB) derived from the data of the experiment represented by curve AB of Fig. 4. As can be seen from the graph, the impedance spike amplitude is greatest at $s=1$–2 mm, after which there is a continuous fall. This regularity was

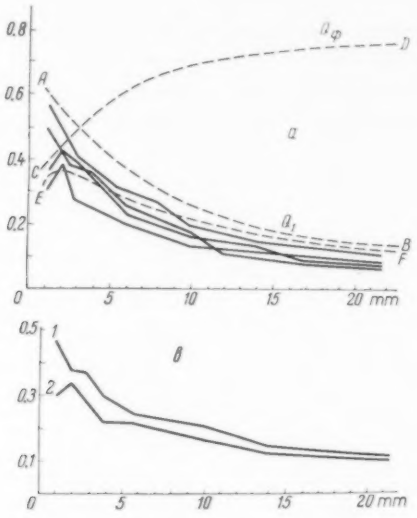

FIG. 5. (a) the continuous, disjointed, lines correspond to the relative change in amplitude of the impedance spikes with increasing interelectrode distance, obtained by direct experiment; (b) change in impedance spike amplitude obtained on one and the same preparation for different values of K; for curve 1, $K<8$ kΩ, for curve 2, $K>12$ kΩ. Ordinates—relative values of maximum impedance spike amplitude; abscissae—distance between impedance electrodes in millimetres.

reproduced, without exception, in each experiment. Photographs of one such experiment are shown in Fig. 6. The fall in impedance spike amplitude with distance indicates that elements connected with the sheath component form the substrate which determines the change of electrical conductivity on excitation. It could have been anticipated that the experimental curves corresponding to the function Q_m would be somewhat below the theoretically constructed curve Q_1, since $Q_m=Q_1-\Psi(s)$.

It is necessary to point out that the value of the contact resistance (the constant K) has some influence on the character of the experimental curves, especially for small s as should be clear from formula (3). Thus, on inspection of the curves AB and EF, Fig. 5(a), obtained from the same experimental curve AB (Fig. 4) and the corresponding functions Q_1, it is clear that on increasing the value of K the maximum values of the impedance spike shift to the right. By comparing the two experimental curves (1 and 2, Fig. 5), obtained on one and the same preparation, it is also clear that an artificial increase in the contact resistance, by connecting an extra resistance

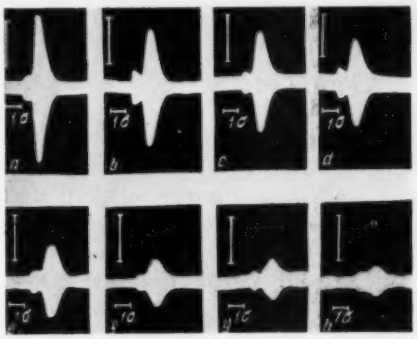

FIG. 6. Photorecording of impedance spikes for various distances between the impedance electrodes. The length of the vertical lines corresponds to the broadening of the oscillograph beam at the instant of "out-of-balance" due to a change of the original resistance by 0·05 per cent. (a), (b), (c), (d), (e), (f), (g), (h)—distance between electrodes equal to 1, 2, 3, 4, 6, 10, 14, 22 mm, respectively. The slight broadening at the beginning of the effect is an artefact.

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in series with the electrode, shifts the maximum values of the impedance spike from $s=1$ mm to $s=2$ mm.

Thus, movements of the maxima of the impedance spike in the range 1–2 mm are due to variations in the magnitude of K for different preparations.

ANALYSIS OF IMPEDANCE SPIKE SHAPE WITH INCREASE OF INTERELECTRODE DISTANCE

Analysis of the change in shape of the impedance spike with increasing interelectrode distance can help to decide in which structures of the nerve fibre the change of electrical conductivity on excitation takes place.

As has already been pointed out above, for the case in which the structures determining the change in electrical conductivity on excitation are in the sheath component, the changes in conductivity of these structures in the neighbourhood of the electrodes are the most important for the recording of the impedance spike. There should be two maxima on recording the impedance changes, one of them connected with the passage of the wave of excitation under the first electrode and the second on passage of the wave of excitation under the second electrode.

When the two electrodes are placed close together, the two maxima, due to the effect of the decrease of resistance on excitation under the first and under the second electrode, merge into one impedance spike. However, with increasing distance between electrodes, if this point of view is correct, a second maximum should appear.

If the conductivity changes on excitation are due to the nerve fibre axoplasm itself then, for any interelectrode distance, there should always be only one maximum since all parts of the nerve are equivalent as regards recording of the impedance spike. This is easy to see from simple geometrical analysis of Fig. 3. If the wave of change of impedance on excitation, the spatial distribution of which is represented by ΔABC, occurs, for example, across electrodes DG, then the maximum of the recorded impedance spike during the passage of the wave will be determined by the instant of greatest area of the ΔABC between the perpendiculars erected from the

points D and G. Such a maximum area, for any distance s, will occur only once—at the instant indicated in Fig. 3.

For experimental investigation of impedance spike shape it was necessary to work with large sweeps of the oscillograph. All the experiments carried out in this series undoubtedly indicated the development of a second maximum in the impedance changes on excitation. The second maximum became sufficiently marked under our recording conditions on increasing the distance between electrodes to 10–12 mm. A photograph of one of the experiments of this series is presented in Fig. 7.

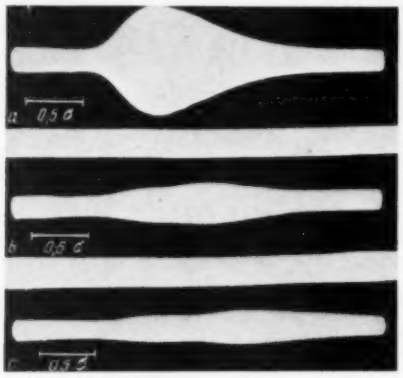

FIG. 7. Changes in shape of impedance spikes with increasing interelectrode distance. (a), (b), (c)—distance between electrodes equal to 2, 10, 12 mm, respectively.

For an interelectrode distance of 2 mm (Fig. 7(a)) only one maximum is observed. In Fig. 7(b) and (c) a picture of the impedance spike when the interelectrode distance is increased to 10 and 12 mm, respectively, is shown. We see, in the first place, a substantial decrease in the amplitude of the impedance changes and, in the second place, the development of a second maximum which is more clearly developed at 12 mm than at 10 mm interelectrode distance. The second maximum cannot be due to dispersion taking place because of differences in speed of conduction of excitation in different fibres, since it appears only on increasing the distance between the impedance electrodes and does not depend on the distance apart of the stimulating electrodes. Calculations show that the second maximum arises, in time, at that instant when the greatest amplitude of the impedance spike should pass under the second electrode.

We think that this fact also points to the active role of the surface structures of the nerve fibre, and not of the axoplasm, in the determination of the impedance changes on excitation.

DISCUSSION

Rushton's formula [13], from which we started our investigation, is derived for the condition of no capacitative elements in the nerve trunk. But since we used alternating current (35 kc/s) for the measurement of electrical conductivity on excitation then, clearly, a capacitative component of the impedance spike, which was not taken into account in the calculations, as well as an active component, must also enter into the observed changes of electrical conductivity. However, taking into

account work in which it has been shown that the capacitative component of the impedance hardly changes with increasing interelectrode distance [16], and also the circumstance that we used not absolute but relative changes in amplitude of the impedance spike, we think that our basic conclusions remain valid.

The original premise for our work is the experimental curve characterizing the change in nerve resistance with increase of interelectrode distance. Analysis of this curve and morphological information on the nerve structure led Rushton to his idea of the cable structure of the myelinated nerve fibre in which the myelin sheath is the poorly conducting insulator and the good conducting core is the axoplasm. At the present time this hypothesis of the "cable" structure of the nerve fibre is essentially the only one which satisfactorily describes the distribution of current in a nerve. At the same time this idea does not, by itself, inevitably lead to recognition of the existence of an active membrane. Therefore, while basing our investigations on the "cable" structure of the nerve fibre, we did not take any definite point of view on the question of the active elements of excitation. However, such a structure of the nerve fibre allows us to analyse two components in the total resistance of the nerve trunk and this, in its turn, makes possible the determination of those elements which are active on excitation.

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From the fundamental conclusion that the structures which are concerned with changes in electrical conductivity on excitation are in the sheath component of the total resistance of the nerve, the following basic consequences flow. These structures (1) possess considerably greater resistance than the axoplasm since the current through them passes only in the radial direction; (2) occupy a surface position in relation to the axoplasm, otherwise their resistance would not appear in the sheath component of the resistance; (3) are active and on excitation their resistance falls. Such a characterization wholly corresponds to the ideas of a membrane which arose in connexion with work on the "passive" and "active" electrical properties of the nerve fibre [2, 3, 9]. Thus, in fact, the membrane resistance enters into the sheath component of the nerve resistance, as should have been expected from the membrane theory. The comparatively small impedance changes which are recorded on excitation in a complete myelinated nerve (0·05–0·1 per cent) are chiefly connected with the fact that the myelin sheath forms the major part of the sheath component of the resistance.

The axoplasm, judging by our results, does not change its resistance on excitation and is clearly not a direct participant in the conduction of excitation processes.

Thus, our experimental results can be explained on the basis of the membrane theory of excitation as applied to the myelinated nerve fibres of vertebrates.

CONCLUSIONS

(1) On the basis of the theory of the change of resistance of a nerve trunk as a function of distance, two curves were obtained, theoretically, of the change of the impedance spike; one of these corresponded to the concepts of the membrane theory of excitation (curve AB, Fig. 2) and the other to the concepts of the phase theory of excitation (curve CE, Fig. 2).

The membrane theory of excitation predicts that the impedance changes should decrease with increasing interelectrode distance because the contribution of the sheath component to the total resistance of the nerve falls. From the point of view of

the phase theory of excitation the impedance changes should increase with increasing interelectrode distance because the contribution of the resistance of the axoplasm to the total resistance of the nerve grows.

(2) The experimental facts quite definitely showed that the impedance spike decreases with increasing interelectrode distance, and the nature of its decrease, within the limits of experimental error, corresponds to the curve obtained theoretically on the basis of the membrane theory of excitation.

(3) When the shape of the impedance spike was studied as a function of increasing interelectrode distance, the appearance of a second maximum was noted. This fact directly stems from the concepts of the membrane theory of excitation, whereas, from the point of view of the phase theory of excitation, only one maximum should occur for any interelectrode distance.

Translated by J. DAINTY

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ON CURRENT AND VOLTAGE IN ELECTROPHYSIOLOGICAL EXPERIMENTS*

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(*Received* 4 *April* 1957)

A METHODOLOGICAL error which is tolerated in electrophysiology has recently been pointed out [1]; in experiments on electrical excitation of nerves the form of the voltage curve is given [2], while from the point of view of the ionic theory of excitation (which preserves, in spite of its imperfections, widespread importance) the physiological effect is determined by the current and not by the voltage. The appearance in

* Biofizika **2:** 5, 614–616, 1957 [Reprint Order No. BIO 80].

tissues of an electromotive force of polarization, depending on the current in a complex manner, can severely distort the shape of the current curve, even in simple cases. The presence of inductance and capacitance in the circuit can considerably increase these distortions. Although this consideration had been noted and partially proved experimentally some time ago [3, 4] and is admitted to some degree [5], yet it is clearly useful to give a more detailed, objective, demonstration of its correctness. For this purpose the simplest possible scheme was utilized (Fig. 1). The investigated

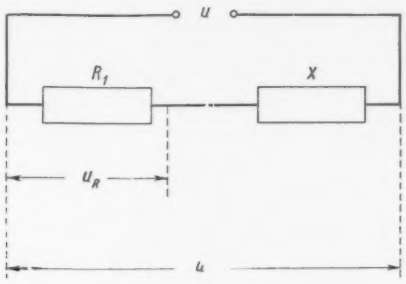

FIG. 1.

object X (part of the wrist, forearm or palm) was connected in series with a resistance R_1, and a source of potential u which gave a curve of a definite shape. The voltage u supplied to the circuit and also the voltage across the resistance R_1, which is proportional to the current i through the object, were applied to an oscillograph; the picture obtained on the oscillograph screen was photographed.

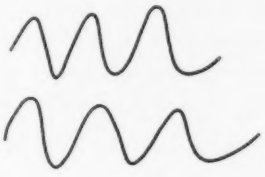

FIG. 2.

Fig. 2 illustrates oscillograms of current (lower) and voltage (upper) when supplying the circuit with a sinusoidal voltage of frequency 100 c/s. Both curves are practically identical in shape. In Fig. 3 oscillograms are given for a frequency of 30 c/s; the middle curve corresponds to a weaker current than the lower. Here the distortions are already quite noticeable.

Fig. 4 illustrates oscillograms for a frequency of 18 c/s for a current which causes noticeable sensation. The distortions in shape of the current are very considerable.

Fig. 5 depicts oscillograms of voltage and current on supplying the circuit with rectangular voltage pulses at a frequency of 3500 c/s and of length 10 μsec. Here the difference in shape between voltage and current is comparatively small since with such rapid processes the polarization is quite insignificant. However, for slow processes its influence becomes much stronger; this is illustrated by the oscillograms of voltage

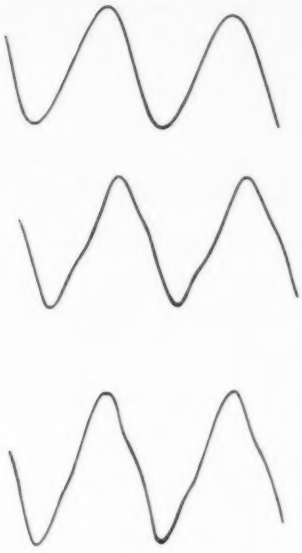

FIG. 3.

and current for slow rectangular pulses (frequency 50 c/s, length 7500 μsec) depicted in Fig. 6.

Finally, triangular pulses, given by a "faradization" apparatus, were studied (Fig. 7).* Thanks to the application of a thyratron circuit, the voltage rises abruptly and then changes according to the law $u = U_m \sin(314t + 2\pi/3)$. In this case the pulse frequency was 100 c/s and the length about 3300 μsec. Here also a reverse current was clearly seen, due to the influence of the polarization e.m.f. It is not difficult to multiply the number of such examples. Confining ourselves to the given examples, we stress that the effects observed are easily explained by the ionic theory of excitation.

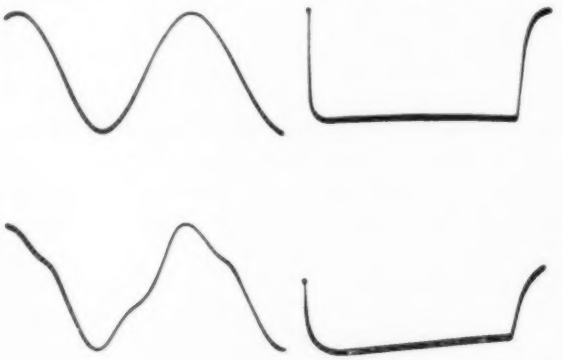

FIG. 4.

FIG. 5.

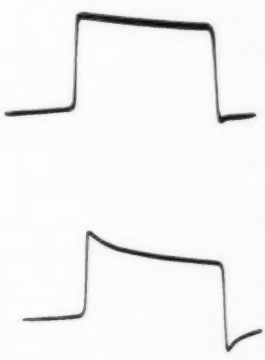

FIG. 6.

* This apparatus was obligingly lent by Prof. I. A. Abrikosov and Docent V. N. Darkshevich. Independently of the present work, these people have carried out similar observations and fully share the point of view developed here.

Assuming, with Nernst and Lazarev [6, 7], that in the object there arises a polarization e.m.f. E, opposing the applied voltage u, and calling the total resistance R (the sum of the resistances of R_1 and of the object, Fig. 1), we obtain in accordance with Ohm's Law

$$i\,R = u - E. \tag{1}$$

Further, the changes of the polarization e.m.f. with time are determined by the number of ions flowing (i.e. the current i) and by the back diffusion of accumulated ions; for small currents one can take the latter to be proportional to the effective value of the polarization. We can therefore write

$$\frac{dE}{dt} = \alpha i - \beta E, \tag{2}$$

where α and β are coefficients which characterize the properties of the object and which are constant in a given experiment.

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Given the law of change of the voltage u, corresponding to one or other case, one can, combining (1) and (2), determine E and hence i. The mathematical aspect of the problem is discussed elsewhere [8]; here we present only the most interesting results.

For small sinusoidal voltages the calculated current is also sinusoidal, but shifted in phase with respect to the voltage. In the experiments this latter circumstance was not checked, but it has been reliably established earlier [9]. On increasing the current, equation (2) loses its validity and the solution of the problem is complicated. However, it was earlier shown [3] that, with a sinusoidal current, the voltage becomes non-sinusoidal and the distortion is more marked the stronger the current and the lower the frequency. Clearly the converse conclusion is correct: if we increase the voltage, preserving its sinusoidal shape, then the curve of current begins to be distorted and this is more noticeable the lower the frequency.

Calculated curves for slow rectangular voltage pulses are depicted in Fig. 8. Finally, calculated curves for the faradic apparatus pulses are presented in Fig. 9.

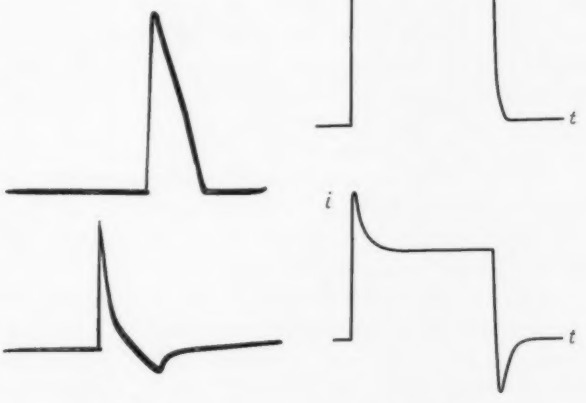

FIG. 7.

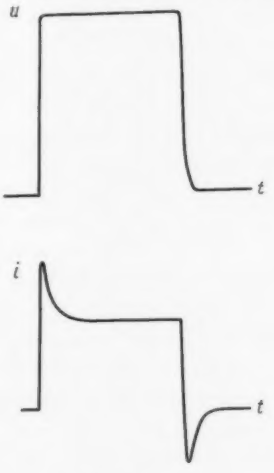

FIG. 8.

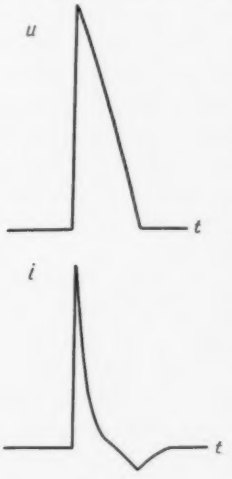

FIG. 9.

Comparison of the calculated curves with the corresponding oscillograms shows that the ionic theory gives satisfactory agreement with experiment. All the material presented here objectively proves the existence of a considerable divergence in shape of the curves of voltage and current.

This shows that it is necessary (in electrophysiological research) to pay special attention to the current, and not to the voltage as is often done at the present time.

Translated by J. DAINTY

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ON BOUND AND FREE SODIUM IN FROG SKELETAL MUSCLES*

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(*Received* 27 *March* 1957)

IN recent years it has been established by many investigators that muscle fibres, and also very varied animal and plant cells, are permeable to all mineral anions and cations, among them sodium [1–5]. It has been found that the sodium in muscle fibres occurs in two conditions—free (in solution) and bound. The bound sodium fraction does not pass from the muscle to a medium deprived of this cation [6–8], and does not exchange with labelled sodium in Ringer's solution [5, p. 287], [8, 9]. It has also been found that the concentration of sodium in isolated frog muscles, immersed in Ringer, increases but still remains a few times smaller than the concentration in the external solution; and this difference in the sodium concentrations is maintained for a long time [6, 10]. In these same conditions the potassium concentration in the muscles stays essentially unchanged [11].

These stated facts are in fundamental contradiction to the conclusions of the classical membrane theory of cell permeability and do not agree with the well-known variant of this theory—Hodgkin's "sodium pump" hypothesis [4]. The facts are better explained from the point of view of the sorption theory of cell permeability [5, 12].

*Biofizika **2:** 5, 617–627, 1957 [Reprint Order No. BIO 81].

This present research is devoted to a study of the distribution of sodium between muscle fibres and the medium surrounding them. The change of sodium concentration in frog muscles with their time of immersion in Ringer has been studied; the amount of free and bound sodium in the muscles has been determined; and the dependence of the sodium concentration in muscle fibres on its concentration in the surrounding medium has been examined.

MATERIAL AND METHODS

All the experiments were carried out on isolated sartorius muscles of autumn and winter frogs (*Rana temporaria*). The sodium concentration in the muscles was determined by the zinc uranyl acetate method [13]. The determination was done immediately after dissecting out the muscles and after various times of immersion in the Ringer's solution. A Ringer's solution of the following composition was used (mM): NaCl–111·11; KCl–3·34; $CaCl_2$–2·70; $NaHCO_3$–2·38.

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The amounts of free and bound sodium were determined from the distribution of radioactive sodium between the muscles and the Ringer. For this the muscles were placed for various times in a Ringer's solution in which part of the NaCl was replaced by ^{24}NaCl. After definite periods of time the muscles were withdrawn from the radioactive solution, dried and ground to a powder in an agate mortar, and approximately 15 mg of this powder was placed on a brass disk for counting. Under similar conditions the number of counts from the deposit was measured after evaporation of 0·1 ml of the radioactive Ringer's solution in which the muscles had been. For convenience, all the disks were covered by aluminium foil. The coefficient (Q) of distribution of ^{24}Na between the muscles and the radioactive salt solutions was determined from the following relation:

$$Q = \frac{\text{number of counts/min in 1 g wet weight of muscle}}{\text{number of counts/min in 1 ml of external radioactive solution}}$$

The concentration of muscle sodium, replaced by labelled sodium from the surrounding solution, was calculated from the formula $C = C_s Q$ where C is the concentration of exchangeable sodium in the muscles and C_s is the sodium concentration in the radioactive solution.

In order to examine the relation between the concentration of free sodium in muscles and the concentration of this cation in the medium, the muscles were placed in Ringer's solutions in which various amounts of sodium chloride (with ^{24}NaCl) were replaced by the corresponding number of moles of sucrose. Then the coefficient Q was determined and from it the concentration of free sodium in the muscles was calculated.

EXPERIMENTAL RESULTS

(1) *The change of sodium concentration in muscles during their immersion in Ringer's solution*

The experimental results of this part of the work are given in Table 1. It follows from this table that the sartorius muscle of the frog, before immersion in Ringer's solution, contains 2·29 $\pm$ 0·17 m.equiv per cent sodium. Similar results have been obtained earlier by many authors [6, 9, 10, 14–16].

TABLE 1. CHANGE OF SODIUM CONCENTRATION IN FROG SARTORIUS MUSCLES AS A FUNCTION OF THEIR TIME OF IMMERSION IN RINGER'S SOLUTION AT A TEMPERATURE OF 18–20°C

Number of experiments	Time of immersion in Ringer's solution	Na concentration in muscles in m. equiv. in 100 g tissue wet weight (M ± m*)	Water content of muscles (per cent)
21	0 (norm)	2·29 ± 0·17	80
6	10 min	2·30 ± 0·34	81
12	30 min	2·67 ± 0·25	81
12	2 hr	3·38 ± 0·33	80
6	3 hr	3·53 ± 0·55	79
12	5 hr	3·85 ± 0·55	80

* M = arithmetic mean; m = mean square error of the arithmetic mean.

After the muscle had been in the Ringer's solution for 2 hr its sodium concentration had increased by 1·09 m.equiv per cent, but during the next 3 hr it had increased by only 0·47 m.equiv per cent. A similar picture of the entry of sodium into frog muscles from Ringer's solution was also observed by Fenn *et al.* [6] and Carey and Conway [10]. The amount of water in the muscles remains unchanged all the time.

(2) *The free and bound fractions of sodium in muscles*

Curves (A) and (B) of Fig. 1 show the time change of the coefficient of distribution of radioactive sodium, Q, between muscles and radioactive Ringer's solution. Curve (A) relates to muscles placed in radioactive solution immediately after dissection, and curve (B) to muscles which have first been in radioactive Ringer's solution for 1·5 hr. It is clear from these curves that in both cases most of the radioactive sodium penetrates into the muscle in the first hour and subsequently the radioactivity of the muscles increases very slowly.

After 2·5 hr the curves (A) and (B) run together in one line which then proceeds wholly parallel to curve (C) which gives the change in the ratio of the concentration

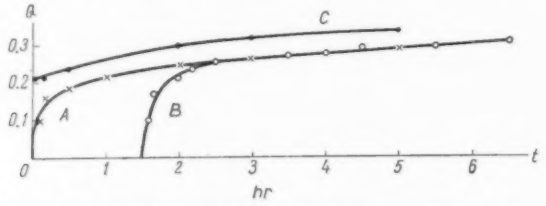

FIG. 1. Curves of change with time of the ratio of the concentrations of ^{24}Na and ^{23}Na in muscles to their concentrations in Ringer's solution: (*A*)—for muscles immersed in radioactive Ringer's solution immediately after dissecting out; (B)—for muscles immersed in the same solution 1·5 hr after dissecting out. For curves (A) and (B),

$$Q = \frac{\text{no of cts/min in 1g wet weight of muscle}}{\text{no. of cts/min in 1ml of Ringer's solution}}$$

For curve (C),

$$Q = \frac{\text{conc. of } ^{24}Na \text{ in muscles}}{\text{conc. of } ^{24}Na \text{ in Ringer's solution}}$$

of total sodium in the muscles, determined chemically, to its concentration in the medium. If all the muscle sodium were free, then in the course of time complete mixing of the muscle sodium with the labelled sodium of the medium should have occurred and then curves (A) and (B) would coincide with curve (C)—which definitely does not happen. This indicates that part of the sodium exists in muscles in the bound state and cannot freely exchange with the sodium in the medium. As we shall see later, this part of the muscle sodium does exchange nevertheless, but extremely slowly.

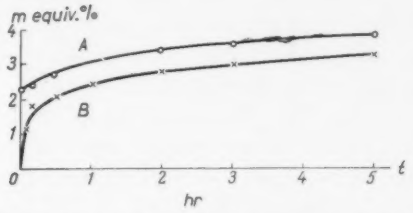

FIG. 2. Curves of the dependence of the sodium concentration (in m.equiv per cent) in muscles on the duration of their stay in Ringer's solution. (A)—change in concentration of total sodium (bound + free); (B)—change in concentration of muscle sodium replaced by labelled sodium of the Ringer's solution.

In Fig. 2 curve (A) shows the change of concentration of total muscle sodium as a function of time of immersion in Ringer's solution, determined chemically (data of Table 1), and curve (B) shows the change, under the same conditions, of the concentration of replaced sodium, calculated from the value of Q given in Fig. 1 (curve (A)). In Fig. 1 it is clear that at first curve (B) rises steeply upwards and then, slowly rising, goes almost parallel to curve (A). After 30 min the difference between the total and exchangeable sodium falls to approximately 0·7 m.equiv per cent and subsequently (up to the fifth hour) changes very little.

The data presented in Fig. 3 support this. The curve in this figure shows the change with time of the fraction of the muscle sodium not replaced by sodium from the medium and found from the value of Q. As is clear from the course of the curve, after the first hour the fraction of unexchanged muscle sodium falls to 20 per cent of the original level and after the next 4 hr decreases in all by 3 per cent.

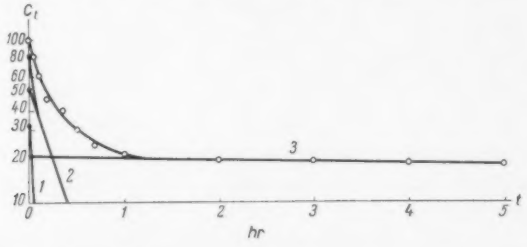

FIG. 3. Change with time of the concentration of muscle sodium unexchanged for labelled sodium of the Ringer's solution. Ordinate—concentration of unexchanged sodium in the muscles in per cent of original concentration (C_t on a logarithmic scale); abscissa—time (t in hr). The curve with the circles shows the dependence of log C_t on t; the straight lines (1), (2) and (3) are the exponential components into which this curve can be split.

If the sodium existed in the muscles as a single fraction of some kind (e.g. in the dissolved state in the extracellular spaces), then the decrease in the concentration of muscle sodium, not replaced by labelled sodium from the Ringer, would be subject to the equation $C_t = C_0 \exp(-kt)$ where C_0 is the initial concentration of unsubstituted sodium (at $t=0$); C_t is the concentration of unreplaced sodium after time t; and k is the constant characterizing the speed of decrease of the concentration of unreplaced sodium.

However, the experimental curve, presented in Fig. 3, indicates a more complex relation. This curve can be split into three straight lines, its exponential components, which suggests the existence of three different sodium fractions in the muscles. In Fig. 3 the straight lines (1), (2) and (3) have intercepts on the ordinate numerically equal to the corresponding initial sodium concentrations in the first fraction (C_1), the second (C_2) and the third (C_3). The tangents of the angles which the straight lines (1), (2) and (3) make with the abscissa are equal to k_1, k_2 and k_3 respectively. Thus, the equation of the curve giving the dependence of C_t on t has the form:

$$C_t = C_1 \exp(-k_1 t) + C_2 \exp(-k_2 t) + C_3 \exp(-k_3 t).$$

The values of the constants of this equation are given in Table 2.

TABLE 2. CONSTANTS FOR THE EXCHANGE OF FROG SARTORIUS MUSCLE SODIUM WITH SODIUM OF THE RINGER'S SOLUTION

Fraction of muscle sodium	Initial concentration of unreplaced sodium		k (hr^{-1})	Time of half-replacement of muscle sodium by Ringer's sodium ($T_{\frac{1}{2}}$ in min)
	(per cent)	(m.equiv. per cent)		
C_1	30	1·05	$k_1 = 23{\cdot}8$	1·8
C_2	50	1·75	$k_2 = 3{\cdot}89$	12
C_3	20	0·70	$k_3 = 0{\cdot}032$	1300
C_0 (total Na)	100	3·50	—	—

From the data of Table 2 it is clear that the fraction C_3 exchanges with the labelled sodium of the Ringer's solution with a speed approximately 700 times smaller than the sodium fraction C_1 and 100 times smaller than the sodium fraction C_2. To all appearances the fraction C_1 represents the sodium of the extracellular spaces, the fraction C_2 the sodium in solution in the muscle fibres and the fraction C_3 bound sodium. It is interesting that a calculation of the size of the extracellular space of the sartorius muscle from the sodium fraction C_1, gives a value of 9·5 per cent of the total volume of the muscle. Determination of the size of this space by other methods ("inulin space", "sulphate space", etc.), carried out by a number of authors [5, p. 207], gives a similar value (9–12 per cent).

These facts indicate that the frog sartorius muscles contain about 0·7 m.equiv per cent of bound sodium, an amount which practically does not change during a prolonged immersion in Ringer's solution. The increase in the muscle sodium concentration in these conditions takes place because of the increase of the free sodium fraction.

(3) *The dependence of the concentration of free muscle sodium on the concentration of this cation in the medium*

Isolated frog sartorius muscles were immersed in sucrose–Ringer's solution having the following sodium (with ^{24}NaCl) concentrations (in m.equiv per cent): 11·11 (without sucrose); 8·33; 5·56; 2·78 and 1·39. In such solutions the muscles, according to their external appearance, excitability, length of life and water content, were no different from muscles immersed for the same time in the usual Ringer's solution.

Before immersion in these solutions the muscles were left for 2 hr in non-radioactive Ringer. After this time (see curve (A), Fig. 2) the sodium concentration in the muscles was practically unchanged on further immersion in this solution. It is important to keep this in view since, for the determination of the concentration of free muscle sodium by the distribution of ^{24}Na, and for the development of the above-indicated dependence, it is necessary to compare the radioactivity of muscles which have been in salt solutions of various sodium concentrations after the onset of diffusion equilibrium between the labelled sodium of the external solution and the muscles.

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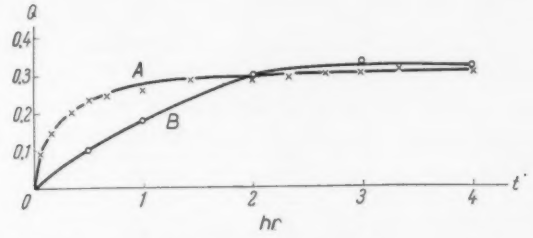

FIG. 4. Curves of the change with time of the ratio of the concentrations of radioactive sodium in muscles to its concentrations in Ringer's solution (Q); (A)—in the usual Ringer's solution with admixture of ^{24}Na, (B)—in Ringer's solution + sucrose with admixture of ^{24}Na (concentration of NaCl = 13·9 mM/*l*).

From the curves of Fig. 4 it is clear that such an equilibrium for muscles in normal Ringer's solution (without sucrose) sets in after 1·5 hr (curve A); but in Ringer's solution with 1·39 m.equiv per cent sodium it sets in after 2·5 hr (curve B). The greater time of onset of equilibrium in the latter case is explained by the fact that not only does exchange take place between labelled sodium of the medium and unlabelled sodium of the muscles, but also the concentration of this cation in the muscles decreases. It is evident that diffusion equilibrium for solutions with intermediate concentrations will set in at intermediate times.

In the following series of experiments the concentrations of free sodium in muscles having been for 3 hr in Ringer's solutions with various sodium chloride concentrations, were determined. During this time, as is clear from the curves of Fig. 4, diffusion equilibrium occurs in all the experimental solutions. The results of this series of experiments are presented in Table 3.

The sodium concentrations in the muscles are calculated on the basis of tissue wet weight and intracellular water. In the latter case the calculation is carried out according to the formula $C=C_s(Q-0{\cdot}1)/0{\cdot}7$ where C is the concentration of free sodium in muscle fibre water (in m.equiv per cent); C_s is the sodium concentration

in the medium (in m.equiv per cent); and Q is the coefficient of distribution of ^{24}Na between muscles and medium.

For the calculation it is assumed that out of 1 g fresh weight of muscle, 0·7 g constitutes muscle fibre water and 0·1 g extracellular space water [5, p. 207].

TABLE 3. DISTRIBUTION OF FREE SODIUM BETWEEN FROG SARTORIUS MUSCLES AND RINGER'S SOLUTIONS WITH VARIOUS CONCENTRATIONS OF SODIUM CHLORIDE AT A TEMPERATURE OF 18–20°C

Number of experiments	Concentration in bathing solution (m.equiv per cent)	Q	Concentration of free sodium in muscles (m.equiv per cent)	
			In 100 g tissue wet weight	In 100 g muscle fibre water
4	11·11	0·31	3·44	3·33
9	8·33	0·31	2·58	2·49
5	5·56	0·32	1·78	1·74
5	2·78	0·33	0·92	0·91
5	1·39	0·35	0·49	0·50

It can be seen from Table 3 that, when the sodium concentration in the medium is decreased eight times, Q increases from 0·31 to 0·35. If this change in Q is neglected, it can be concluded that the concentration of free sodium in the muscles changes in direct proportion to the change in concentration of this ion in the bathing equilibrium solution.

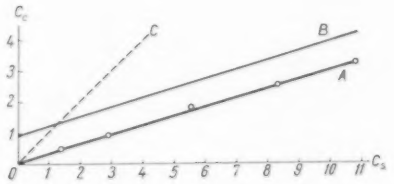

FIG. 5. Dependence of sodium concentration in muscle fibres on its concentration in the medium. Ordinate—concentration of sodium in muscle fibres (C_c, m.equiv in 100 g intracellular water); abscissa—sodium concentration in surrounding solution (C_s, m.equiv).

This dependence is well illustrated graphically. In Fig. 5 the straight line (A) shows the dependence of the concentration of free sodium in the muscle fibres, (C), calculated on their water content, on the concentration of sodium in the bathing equilibrium solution (C_s). The straight line (A) corresponds to the equation $C=C_sK$ where K is the coefficient of proportionality, equal to 0·30 in this case.

This indicates that, regardless of the sodium concentration in the medium, its concentration in the muscle fibre water is equal to 30 per cent of the external concentration. We suggest that the exchangeable sodium of the muscle fibres is ionic sodium, dissolved in the muscle fibre water.

If the sodium concentration in the sarcoplasm in conditions of diffusion equilibrium were equal to its concentration in the medium, then we would obtain $K=1$ and then the relation between C and C_s would correspond to the straight line (C).

This is certainly not observed, which indicates, in our opinion, that the sarcoplasm has special solvent properties. We have seen that, as well as dissolved sodium, muscles contain about 0·7 m.equiv per cent unexchangeable sodium. As Fenn *et al.* [6] and Conway and Carey [9] have shown, this sodium does not disappear from the muscle when it is soaked for a long time in sucrose solutions which do not contain sodium. Consequently, and in our experimental conditions, the total quantity of sodium in the muscles (C_c) is made up of bound (A) and dissolved (exchangeable, C_s), and the dependence of C_c on C_s is expressed by the formula $C_c = C_s K + A$.

In Fig. 5 this equation is given by the straight line (B) in which the amount of bound sodium is given not with respect to muscle wet weight (0·7 m.equiv per cent), but with respect to the amount of dry matter which corresponds to 100 g of intracellular water (0·93 m.equiv per cent).

The facts presented here almost fully agree with those obtained by us earlier in experiments carried out by a similar method and with data calculated from the results of the work of Fenn *et al.* [5].

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DISCUSSION

The experimental facts indicate that, in frog skeletal muscles, sodium is in two forms—free and bound. Clearly this phenomenon occurs widely. For example, it has been established by a number of investigators that both free and bound sodium are present in the erythrocytes of various animals [17–19].

Isolated muscles, placed in Ringer's solution, absorb some sodium; this absorption takes place due to an increase in its free fraction. Side by side with the entry of sodium under these conditions, chloride ions also enter the muscle [6, 10]. Isolated nerves and single giant fibres of cephalopod molluscs, placed in artificial salt media, also absorb noticeable amounts of sodium and chloride [4, 20]. To all appearances this phenomenon is due to the absence, in the artificial salt media, of colloids. Thus, according to the observations of Fenn *et al.* [6] and Carey and Conway [10], isolated frog muscles, immersed in the plasma or blood of this animal, do not change their sodium concentration. No change in chloride concentration occurs in isolated muscles immersed in blood. The experiments of Hegsted *et al.* [21] support this. They showed that if rats are fed on a non-protein diet, then the quantity of protein in their blood plasma greatly decreases and at the same time the sodium and chloride concentrations in the muscles increase.

Some authors [6, 20, 21] assume that the increase in sodium and chloride concentrations in muscles immersed in Ringer's solution is due to an increase in the size of the extracellular spaces. We think such an explanation impossible because the amount of water in muscles in Ringer's solution does not change. Also opposed to this explanation is the fact that the single giant nerve fibres of some cephalopods, which do not have extracellular spaces, when placed in sea-water or artificial salt media also absorb a certain amount of sodium and chloride.

As the experimental facts show, practically all the free sodium of the muscles exchanges with the sodium of the medium in 40–60 min; nevertheless the sodium concentration in the muscle fibres, although it increases during the muscles' immersion in Ringer's solution, still remains 3–3·5 times lower than in the surrounding solution.

At the present time, most investigators who try to explain this fact assume the presence in the membrane of a special enzyme mechanism which continually expels

from the cells the sodium which enters them (in this connexion the expressions "extrusion" or "pumping" of sodium are used) [2, 4, 22, 23]. Hodgkin [4] has called this suggested mechanism the "sodium pump". In Hodgkin's opinion the sodium pump acts so as to create the impression that muscle and nerve fibres are completely impermeable to sodium; hence conditions arise in which one can apply Boyle and Conway's hypothesis [16] about the Donnan membrane distribution of ions between muscle fibres and the medium surrounding them.

However, very recently a number of investigators have shown that the sodium pump hypothesis is in serious contradiction with many facts [5, 24, 25]. Thus, the pump hypothesis issues from the fact that all the potassium exists in the protoplasm in the free (ionic) state. But this is not so. Many investigators have shown that the potassium in the protoplasm is chiefly in the bound (non-ionic) state [5, 8, 11, 12, 15, 24, 25].

Further, according to Hodgkin, the increase in sodium concentration in muscle and nerve fibres should be accompanied by an equivalent decrease in their potassium concentration.

According to the observations of a number of authors, in many cases this is not observed [5, 24]. Harris [11] found that frog sartorius muscles immersed in Ringer's solution lost hardly any potassium in 24 hr, while the results of our experiments and the observations of other authors [6, 10] show that under these conditions the sodium concentration in the muscles almost doubles in 5 hr.

The above observations of Harris are also fully supported by the results of our experiments. By the cobalti-nitrate method we have studied in detail the change in potassium concentration in sartorius muscles during their immersion in Ringer's solution. The results of these experiments are given in Table 4.

TABLE 4. CHANGE IN POTASSIUM CONCENTRATION IN FROG SARTORIUS MUSCLES DURING THEIR IMMERSION IN RINGER'S SOLUTION

(For each time the mean result of analysis of 8 muscles is given. Temperature 18–20°C)

Duration of immersion of muscles in Ringer (hr)	Potassium concentration in muscles (m.equiv per cent)	
	per 100 g tissue wet weight	per 100 g intracellular water
0	8·72	12·41
1	8·66	12·32
3	8·95	12·74
6	8·23	11·71
15	8·77	12·48
21	8·44	12·01
25	8·18	11·64
47	5·56	7·89

It has been shown above that the distribution of the dissolved sodium fraction (C) between muscle fibres and surrounding medium (C_s) is subject to Henry's Law: $C=C_sK$. According to our determinations, the coefficient K in this equation is equal to 0·30 for sodium. This indicates that the concentration of dissolved sodium in the

muscle fibres under conditions of diffusion equilibrium is 70 per cent smaller than in the medium (regardless of its concentration in the latter).

It was earlier [5] established that the distribution between cells and medium of various non-electrolytes, large organic electrolytes and many mineral ions is subject to this law, and the magnitude of the coefficient K for a series of substances varied between 0·25 and 0·45. If the solubility of non-electrolytes and other substances in the sarcoplasm is so strongly decreased, then there is no reason for not also assuming that the solubility of sodium in it will be lower than in the surrounding medium. We therefore consider that there is no necessity to assume the existence of a sodium pump mechanism.

According to our ideas, protoplasm has the properties of a "phase" and in consequence of this, all the water in it is bound in the same way as it is bound in coacervates. To this is due the lowered concentration of dissolved sodium in muscle fibres as compared with its concentration in the medium.

The predominance of the cell potassium concentration over its concentration in the medium is wholly due to its bound (non-ionic) fraction [5, 12, 15, 24–26].

As we have seen, the absorption of sodium by muscles in Ringer's solution takes place because of the increase of its dissolved fraction; in other words the solubility of sodium in sarcoplasm under these conditions increases, which leads to a growth of the magnitude K from 0·1 (in the normal) to 0·3 (after a stay in Ringer). The cause of this clearly lies in the unfavourable effect of the artificial, pure, salt solutions on the protoplasm.

When a cell is severely and irreversibly injured this coefficient, as is well known, becomes unity (the concentrations of sodium in the cell and the medium are equal).

In conclusion I express my deep gratitude to L. N. Pisareva for help in carrying out the experimental part of this work.

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SUMMARY

(1) The change in sodium concentration in isolated sartorius muscles of the frog (*Rana temporaria*) during their immersion in Ringer's solution was studied by the zinc uranyl acetate method. Using ^{24}Na the speed of replacement of muscle sodium by sodium in the medium was measured and the relation between the concentration of sodium in the muscle fibres and its concentration in Ringer's solution was determined.

(2) Before immersing them in Ringer the sartorius muscles contained $2{\cdot}29 \pm 0{\cdot}17$ m.equiv per cent sodium; 2 hr after immersing them in this solution they contained $3{\cdot}38 \pm 0{\cdot}33$; and 5 hr after they contained $3{\cdot}85 \pm 0{\cdot}55$ m.equiv per cent. Under these conditions the amount of water and the potassium concentration did not change in the course of 24 hr.

(3) Sodium exists in muscles in two forms—bound and free. Bound muscle sodium is about 0·7 m.equiv per cent. The increase in muscle sodium concentration during their stay in Ringer's solution is due to an increase in the free (dissolved) sodium fraction. The exchange of free muscle sodium for labelled sodium in Ringer takes place in approximately 40–60 min; but the exchange of bound (non-ionic) sodium takes place at a rate 700 times smaller than the rate of exchange of the sodium

of the extracellular spaces, and 100 times smaller than the rate of exchange of the free sodium of the muscle fibres.

(4) The dependence of the concentration of free sodium in the muscle fibres (C) on its concentration in the medium (C_s) is subject to Henry's law: $C=C_sK$. The value of K in this case is equal to 0·30.

(5) The data obtained in this work agree well with the conclusions of the sorption theory of cell permeability and contradict the membrane theory.

Translated by J. DAINTY

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ELECTRON DIFFRACTION AND ELECTRON MICROSCOPE STUDIES OF CHLOROPHYLL a + b AND GRAMICIDIN S*

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(*Received* 11 *January* 1957)

CHLOROPHYLL a+b and gramicidin S have already been studied with X-rays. This work showed that both amorphous [1] and crystalline [2, 3] chlorophyll can be produced. Electron micrographs show that the chlorophyll microcrystals are small needle-shaped plates commonly rolled into tubes [3]. The X-ray structural data on gramicidin S show the molecule to be a decapeptide [4].

In this work chlorophyll a+b and gramicidin S were studied by electron diffraction and electron microscopy.†

METHODS

The chlorophyll a+b‡ and the gramicidin S§ were deposited from solution on to collodion films (0·75 per cent solution of collodion in amyl acetate). The following chlorophyll solutions were used: 96° alcohol, xylene and petroleum ether. For the solution of gramicidin S, 70° alcohol was used.

A UEM-100 electron microscope was used since diffraction patterns and electron micrographs can be obtained simultaneously from the same area. Check diffraction patterns were taken at the Institute of Crystallography, Academy of Sciences of the U.S.S.R. The camera was of such design that "oblique texture" patterns could be taken at various incident angles of the beam.

Since collodion was used as backing, it had first to be examined as regards its

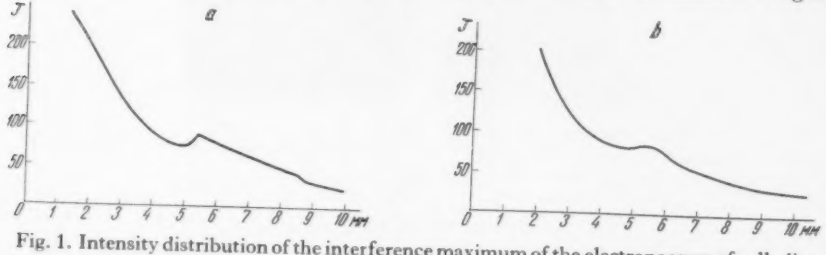


Fig. 1. Intensity distribution of the interference maximum of the electronogram of collodian film.

a—before electron exposure. b—after electron exposure.

† The materials were prepared in the Institute of Biochemistry, Academy of Sciences of the U.S.S.R.: chlorophyll a + b in Professor Krasnovskii's photobiochemistry laboratory and gramicidin S in Znamenskii's microbial biochemistry laboratory.

‡ The material showed the visible absorption spectrum of chlorophyll a + b. Some traces of pheophytin may have been present.

§ The constants were those of gramicidin S (m.p. 268°C, 13 per cent N, etc.).

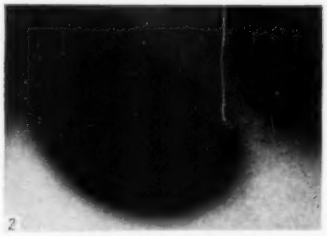
* Biofizika **2**: 5, 628–632, 1957 [Reprint Order No. BIO 82].

reaction to electron bombardment in the electron microscope. The patterns obtained from pure collodion at low emission currents differed from those found after bombardment at higher beam currents. The intensity distributions in the first ring differ between the irradiated and non-irradiated films (Fig. 1).

The effects of vacuum and electron bombardment on the materials were examined by comparing the electron diffraction patterns with X-ray pictures taken with the characteristic radiation of copper.

RESULTS

It was found that good diffraction pictures were not obtainable with these materials when the camera was operated in the usual way because the sharp crystal reflections rapidly vanished. Very low current densities were used to keep the patterns sharp. This was done by reducing the condenser lens and filament heating currents with full negative cut-off bias in the UEM-100. At 60 kV the emission current was 1–2 μA* (without leakage current).



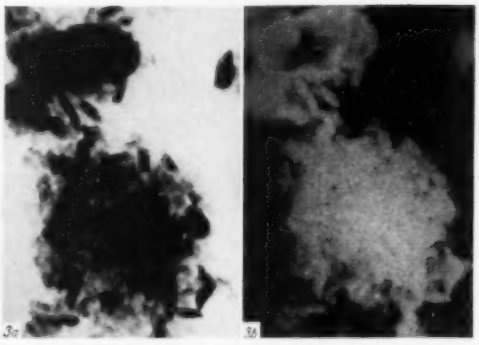


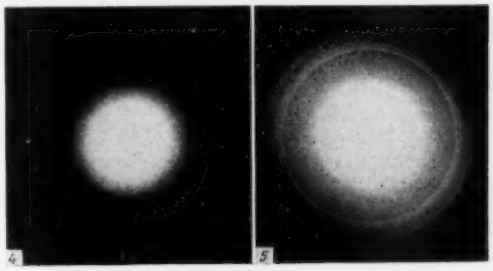
Fig. 2. Microphotograph of Chlorophyll a+b in 96° spirit solution.
Fig. 3. Microphotograph of Chlorophyll a+b in petroleum ether solution. Enlarged 15 times. a—in a light field; b—in a dark field.

Chlorophyll a+b. Crystallization is dependent on the solvent. Crystallization did not occur from alcohol solution (Fig. 2); crystalline particles were present together with a large mass of amorphous material when xylene was used; the best crystallization was found with petroleum ether.† In this case the crystals formed clumps (Fig. 3).

The objects were studied by electron diffraction at the same time. Only diffuse rings (Fig. 4) were found with the alcohol solutions. With xylene solutions the diffuse background was strong and the sharp maximum fell off in intensity with

* The emission current and current through the object are different.
† Chlorophyll a + b does not dissolve completely in petroleum ether or xylene.

increase in scattering angle. The diffuse background was weak with the petroleum ether preparations and the sharp rings gave interplanar spacings the same as for the xylene preparations (Fig. 5). Layer lines were seen when the beam was incident at 60° to the film.



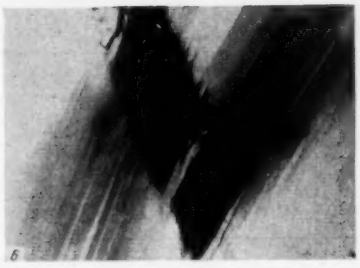


Fig. 4. Electronogram Chlorophyll a+b in 96° spirit solution.
Fig. 5. Electronogram Chlorophyll a+b in petroleum ether solution.
Fig. 6. Microphotograph of oriented gramicidin. Enlarged 15,000 times.

The interplanar spacings computed from the diffraction patterns and the intensities agree with the X-ray data (Table 1).

TABLE 1. ELECTRON DIFFRACTION AND X-RAY DATA FOR CHLOROPHYLL a + b

Mean electron diffraction data for chlorophyll a + b		Mean X-ray data for chlorophyll a + b		Mean electron diffraction data for chlorophyll a + b		Mean X-ray data for chlorophyll a + b	
d	Intensity*	*d*	Intensity*	*d*	Intensity*	*d*	Intensity*
7·80	Strong	7·68	Strong	2·17	Weak		
7·38	Very strong			2·04	Weak	2·05	Weak
4·32	Average	4·22	Average	1·95	Very weak		
4·10	Average	4·06	Average	1·81	Weak	1·83	Weak
4·08	Very weak			1·73	Very weak		
3·84	Very weak			1·67	Very weak	1·62	Weak
3·66	Average	3·66	Average	1·53	Very weak		
2·74	Weak	2·78	Average	1·43	Very weak		
2·70	Weak			1·20	Very weak		
2·43	Weak	2·44	Average				

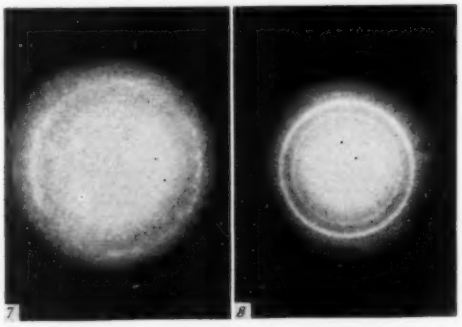
* Pinsker's [5] scale for electron diffraction patterns.

Gramicidin S. Photomicrographs showed that this readily gave orientated crystals forming a texture on the film (Fig. 6). The electron microscope picture and diffraction patterns also corresponded. The orientated specimens gave arcs on the diffraction patterns and the non-orientated rings (Figs. 6, 7). Layer lines were seen with the electron beam incident at 60° to the film. The interplanar spacings computed from the diffraction patterns and the intensities agree with the X-ray data (Table 2). The peaks with d=4·74 Å and 4·59 Å in electron diffraction correspond to one with d=4·62 Å on the X-ray patterns, which may be due to the low resolving power of the small-radius X-ray camera.

TABLE 2. ELECTRON DIFFRACTION AND X-RAY DATA FOR GRAMICIDIN S

Mean electron diffraction data for gramicidin S		Mean X-ray data for gramicidin S		Mean electron diffraction data for gramicidin S		Mean X-ray data for gramicidin S	
d	Intensity*	*d*	Intensity*	*d*	Intensity*	*d*	Intensity*
12·8	Average			4·59	Very strong		
9·33	Average			4·14	Average		
8·51	Average	8·80	Strong	4·07	Weak		Weak
7·32	Very weak	7·32	Very weak	3·73	Weak	3·80	
5·79	Average	5·89	Weak	3·54	Average	3·28	Weak
5·58	Average	5·30	Weak	3·22	Very weak	2·87	Very weak
4·74	Strong	4·62	Strong	2·98	Very weak	2·64	Very weak

* Pinsker's [5] scale for electron diffraction patterns.



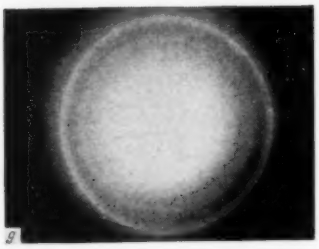


Fig. 7. Electronogram of oriented gramicidin. V=60 sq.
Fig. 8. Electronogram of gramicidin. V=60 sq.
Fig. 9. Electronogram of gramicidin. V=60 sq.

It is clear [6–9] that intense electron bombardment causes the diffraction patterns of macromolecular compounds to change. With ramie, polythene, cellulose hydrate, cellulose, etc. the sharp patterns vanish and three diffuse rings appear.

Electron diffraction studies on the biological objects used here showed them also sensitive to electron bombardment. The following diffraction pattern changes were observed on gradually increasing the emission current. At 2–4 μA the weak large-spacing reflections disappeared in a few seconds. Several close but separate lines combined into one. The intense peaks corresponding to $d=7{\cdot}8$ Å in chlorophyll and $d=4{\cdot}6$ Å in gramicidin are the most stable. At ~8 μA the sharp maxima are replaced by diffuse ones typical of amorphous materials. The gramicidin was the more sensitive. This is clear from the diffraction pictures. The changes may be due to the beam heating the specimen [10]. The special properties of biological objects may result in there being other causes of diffraction pattern change than beam heating.

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The patterns were studied at accelerating voltages of 40, 60, 80 and 100 kV. The ratio of elastic to inelastic electron scattering increases with voltage, and less heat is produced. The diffuse background also becomes weaker at higher voltages [8, 11]. With biological materials it is desirable to reduce the background intensity by using high voltages, as these materials show a sharp intensity fall-off with increase in scattering angle, and the diffuse backgrounds are strong. With our materials, comparison of the diffraction patterns at various accelerating voltages showed that the higher were the better. Thus, the weak 2·89 and 3·22 Å reflections in gramicidin (Fig. 8) can mostly be resolved at 100 kV but are not seen at 40 kV. At 40 kV the patterns are much worse; low-intensity maxima vanish and the diffuse background becomes much stronger (Fig. 9).

It is known [12] that interference maxima can be found at a suitable beam incidence angle in dark-field operation with crystalline materials. Although our materials were crystalline no such maxima were seen at any beam angle (Fig. 2b). The diffraction pattern from this area had become diffuse after dark-field examination. It may be that the high current density used in producing the electron microscope image had heated this area of the specimen too strongly. Heating the crystals destroys the regular internal structure.

SUMMARY

(1) It has been shown that stable diffraction patterns are obtainable from chlorophyll a+b and gramicidin S.

(2) The optimum conditions for doing this in the UEM-100 are accelerating voltage 100 kV, emission current 1–2 μA and minimum exposure time.

(3) The large-spacing weak peaks vanish when the current is raised from 1 to ~4 μA and the sharp maxima are broadened. The diffraction pattern becomes diffuse on increasing the current further.

(4) Diffraction patterns of the oblique texture type are obtained by using an angle of incidence of 60°.

(5) The interplanar spacings computed from the electron diffraction patterns coincide with the X-ray values.

(6) Interference maxima were not seen under dark-field conditions at any incident angle of the beam.

In conclusion I express my indebtedness to Professor A. E. Kriss and to Dr. Yu. M. Kushnir for advice on this work.

Translated by J. E. S. BRADLEY

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RESULTS OF A STUDY OF THE COLOUR VISION OF THE CHINESE OAK SILKWORM*

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(*Received* 14 *March* 1957)

THE question of colour vision in insects is one of the problems of the comparative physiology of sensory organs calling most urgently for attention at the present time. This communication gives the results of the oscillographic registration of the nature of the bioelectrical activity of the abdominal nerve chain ganglia of the Chinese oak silkworm (*Antheraea pernyi* Quer.) in response to various colour stimuli applied to the eye of the insect.

METHOD

The experiments were performed on eyes, prepared together with the isolated ganglionic chain, from Chinese oak silkworm (*Antheraea pernyi* Quer.) imagines. The prepared specimens were placed on glass rollers in a special humid chamber. Registration of the biocurrents transmitted from the second thoracic ganglion was carried out by a Soviet-made loop oscillograph (KhETI) with resistance-capacity coupled balanced input amplifier. The over-all sensitivity of the apparatus was such that it could register potentials not lower than 5 μV in intensity. The frequency characteristic was almost straight over the range 2–15,000 c/s. The light stimuli to the insect's eye came from a 100 V incandescent lamp. The beam of light, after passing through a filter of the appropriate colour, entered the window of the sound-proof, insulated dark chamber containing the specimen. The light was then directed

* Biofizika **2:** 5, 633–636, 1957 [Reprint Order No. BIO 83].

on to the specimen by means of an appropriate lens and mirror arrangement inside the chamber at an angle of 45° to the descending line of the beam. The light-source was at a distance of 115 cm from the eye of the specimen. The intensity of illumination was determined by a light-meter as the beam entered the dark chamber.

Determination of the intensity of the colour illumination was by the calibrated scale of the light-meter. The moment of colour stimulation, recorded on cinefilm by the oscillograph's marker, corresponded to the moment of illumination or obscuration of the eye and isolated nerve chain of the prepared insect.

This series of studies in the comparative physiology of Chinese oak silkworm ganglia was carried out in 1948, in the laboratory of the late Professor S. V. Kravkov at the Institute of Psychology of the RSFSR Academy of Pedagogical Sciences, and with the constant help of S. I. Struchkov.

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RESULTS AND DISCUSSION

The action of colour illumination on the visual analyser of the insect was always followed by a gradual rhythmical response of discharges from the nerve cells of the thoracic ganglion. The data obtained in the course of this direct study of the central nervous system of an insect show that the most effective response is evoked by blue illumination, followed by green and orange-red in that order (see Fig. 1).

In spite of the fact that the intensity of orange-red illumination of the insect eye was 227 lx, i.e. was two-and-a-half or three times as great as that of the blue (90 lx) and green (70 lx) the end result in effective response was only one-quarter as strong for the orange-red as for the blue, and one-third as strong as for the green.

The objection might arise that in the fourth oscillogram (see Fig. 1) the reduction in the response reflex is the result of progressive deterioration in the expressed activity of the nerve cells, so that the effect of orange-red illumination on the eye is not shown here. This objection, however, is easily dealt with by reference to the control oscillogram, no. 5. As this shows, stimulation of the eyes with white light produced a response reflex five times as intense as the action potential for stimulation with orange-red light. Furthermore, oscillogram no. 6 refers to another case (from another experiment), in which stimulation of eyes with orange-red light produced no responding reflex whatever.

We see the cause of such a difference in the effectiveness of the rhythmical discharges from the thoracic ganglion's nerve cells as lying mainly in the photochemical processes which on each occasion unfold in the retina of the eye, i.e. in the peripheral portion of the insect's visual analyser. It would appear that the retina of the specimen studied reacted in a qualitatively different manner to reception of differently coloured illumination.

Comparative analysis of the biocurrents of the rhythmic series of the thoracic ganglion shows that the grading of the response finds expression in a quantity inversely proportional to the spectral wavelength of the illumination applied to the insect's eye.

In these studies we did not have the opportunity of noting the effect of the infra-red (8600–8350 Å), yellow (5850–5750 Å) or ultra-violet (3020–2900 Å) parts of the spectrum. If this gap were to be filled in it would give us a fuller picture of the physiological dependence between reactions of the nerve cells of the thoracic ganglion

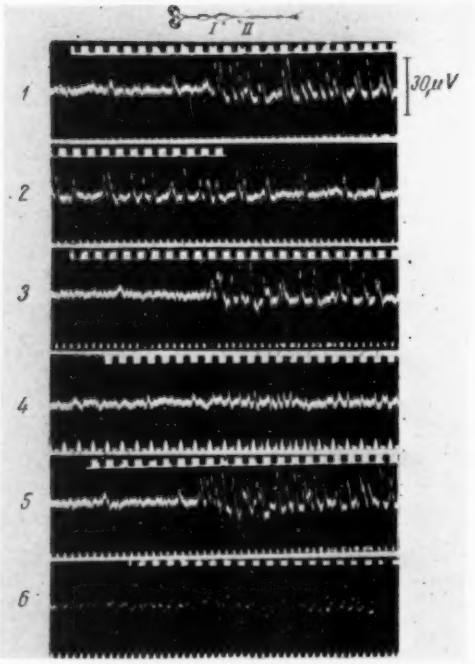

FIG. 1. Change in electrical activity of second thoracic ganglion upon illumination of the eyes with blue, green and orange-red light. The placing of the electrodes is the same in all the cases given here, and is as shown in the sketch at the top. Intensity of colour illumination was determined by a light-meter as the ray entered the chamber. (1)—blue light; (2)—green light; (3) and (4)—orange-red light; (5)—control, white light; (6)—2nd specimen, orange-red light.

and spectral illumination of an insect's eyes. Even these first data in the field, however, enable us to draw the conclusion that the greatest reaction from the visual analyser of the insect comes in a spectral region nearer the violet than the red end of the spectrum.

Apart from the peculiarities in colour vision of the Chinese oak silkworm here described, a comparative analysis of oscillograms 1, 3 and 4 in Fig. 1 reveals without difficulty a difference in the latent periods for the expression of reflexes to different spectral illumination of the eyes. On oscillogram no. 4 it is clearly visible that under the action on the eye of the specimen of orange-red light (227 lx) the reflex response followed after 260 sigm,* i.e. 140 sigm earlier than that for the blue, 120 sigm earlier than that for the green, and 40 sigm earlier than that for the white light (control oscillogram, no. 5).

We consider that the reason for this difference in the latent period in the reflex rhythmical discharges of the nerve cells of the thoracic ganglion lies mainly in the different ways by which excitation spreads from the retina to the second thoracic ganglion. It would seem that when the eyes were illumined with orange-red light, excitations spread from the retina to the thoracic ganglia of the abdominal chain along a path with a smaller number of synaptic interruptions than that followed when blue

* This expression is not explained in the Russian—*Translator.*

or green light was used on the eyes. This is an assumption rather than a matter finally proved. But the fact just quoted of a change in the latent period shows that this depends closely on the spectral nature of the light used on the insect's eyes. We feel that for the comparative physiology of colour vision this is a fact of great interest, needing closer, special, study.

We must here make the proviso that the different forms of reaction by the visual analyser to differently coloured illumination, established above, refer only to the object under study. We have no right to transfer them as rules for all species of insects. Nevertheless, from personal observation of the behaviour of bees we have become convinced that they too react differently to the different sections of the spectrum.

CONCLUSIONS

(1) Illumination of the insect's eyes with light of differing colour composition always evoked a gradual rhythmical discharge of impulses by the nerve cells of the thoracic ganglion. In spite of the fact that the intensity of the illumination used in the case of orange-red light was two-and-a-half to three times as great as that employed for the blue and green light, the amplitude of the fluctuations arising in the second thoracic ganglion under orange-red illumination was only one-quarter of the amplitude of the response to blue illumination, and one-third of that of the response to green illumination of the eyes.

(2) The gradualness of the response is expressed as a quantity inversely proportional to the wavelength of the spectral illumination used to stimulate the eye of the Chinese oak silkworm.

(3) We see the cause of such a difference as lying mainly in the processes occurring in the peripheral portion of the visual analyser of the insect. There is every foundation for the assertion that the eye of the Chinese oak silkworm reacts in a qualitatively different way to differently coloured illumination.

Translated by R. KISCH

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COLOUR DISCRIMINATION THRESHOLDS*

SOME REMARKS IN REPLY TO MacADAM'S PAPER CONCERNING OUR WORK IN COLOUR DISCRIMINATION

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(*Received* 14 *June* 1956)

IN the December 1955 issue of the " Journal of the Optical Society of America ", MacAdam [1] has an article which touches on our work of 1951 on colour discrimination thresholds, which was published in 1953 [2]. He performed laborious and complex calculations to transfer our data to the equal-luminosity plane, assigning particular importance to this.

* Biofizika **2**: 5, 637–641, 1957 [Reprint Order No. BIO 84].

MacAdam sees colour not just as an abstraction but as the result of a special function of vision which is coupled to the function of evaluation in terms of the brightness component.

Our view is different. We assume that the basic physiological axes, which are organically related to the fact of colour being three-dimensional and to the material nature of the retinal receptors themselves, determine the principal semiaxes of the colour discrimination threshold ellipsoids. Then the plane of equal luminosity gives one of the many possible sections of the threshold ellipsoids, but not the principal one.

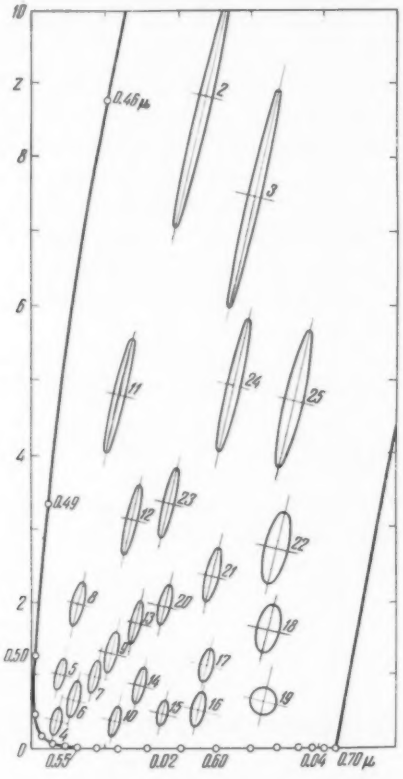

FIG. 1.

Parry Moon [3] has decisively demonstrated the complexities which the equal-luminosity plane introduces; he represents MacAdam's ellipses [4] (Fig. 1) on the (x, z) plane, i.e. in the form in which MacAdam dealt with them in his observations. It is clear why MacAdam could not restrict the measured data to the equal-luminosity plane and was forced to represent his ellipses as projections on *another* plane (x, y). But of course projections of threshold ellipses on any other plane are not adequate as threshold ellipses in the equal-luminosity plane.

Our threshold ellipses [2] are represented directly on the plane in which they were determined on our double three-colour colorimeter, which is typical of normal

three-colour colorimeters. By finding the co-ordinates (a, b, c) of some colour on the three-colour colorimeters we get the red-green co-ordinates (r, g) from the expressions

$$r = \frac{a}{a+b+c} \text{ and } g = \frac{b}{a+b+c};$$

these can also be used on a family of r–g curves [2] which contains our 36 ellipses.

The unsuitability of the equal-luminosity plane may be a cause of the discrepancies between our results and MacAdam's, on which he remarks. Also, the readings for MacAdam's observer (P.G.N.) relate to match settings while ours relate to threshold ones, where the mismatch is still appreciable. This observer also sometimes gives large deviations from others of MacAdam's observers (D.L.M. and W.R.B.) in the shapes and orientations of the threshold ellipses [5]. Some of these are shown in Fig. 2. We see here (in a projection from the equal luminosity plane on to the x–y plane) a case where P.G.N.* and the other two observers give ellipses of very different orientations (nos. 1, 3, 12, 14, 16 and 18). Cases where the ellipses are of different sizes are also found. Those for P.G.N. are sometimes larger than for the others (nos. 8, 11, 13, 25) but there are cases (nos. 4, 19, 27, 33, 39) where the converse is true, P.G.N's being considerably smaller (in general D.L.M. and W.R.B. agree better with one another in ellipse size and orientation).

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These discrepancies indicate that a variety of combinations of colour differentiation acuity with respect to the three physiological axes can occur, and not just in these observers alone.

Meanwhile prior examination of our observers on the anomaloscope [6] (for each physiological axis) enables us to select a homogeneous group with normal colour vision, thereby also obtaining readings of a single species (see figure showing the ellipses in [2]).

In our work of 1951 we did not neglect to compare our results with MacAdam's [4]. We determined the values of g_{11}, g_{12} and g_{22},† and from these were computed (Lobanova) the x–y ellipses for eight of our 36 colours. In Fig. 3 the ellipses computed by MacAdam's method [7] are enlarged by factors of 3 for comparison with our threshold ellipses and the two sets are shown together in the x–y plane.

Comparing ellipse diameters in those directions for which, as MacAdam has shown [1, Fig. 3]‡ this is permissible, we find ratios of from 3 to 1. Such discrepancies are quite natural if we remember the differences in colour vision between the observers and the differences in the criteria and structure of the fields of view.

The differences in other diameters in Fig. 3 are primarily due to the differences in the colour planes used by us and by MacAdam; but all this is compatible with what can be seen in Fig. 2.

In any case we completely reject MacAdam's suggestion [1] that these discrepancies may act as a link between motor and visual impulses, since in our experiments the observer was not responsible for adjusting the filter, as was the case with P.G.N. Our observers only observed.

In order to compare his results of 1942 with ours, MacAdam [1] used relationships

* Dotted.

† See [7, p. 18]. The author estimates the accuracy as ±30 per cent.

‡ These directions are shown dashed in Fig. 3.

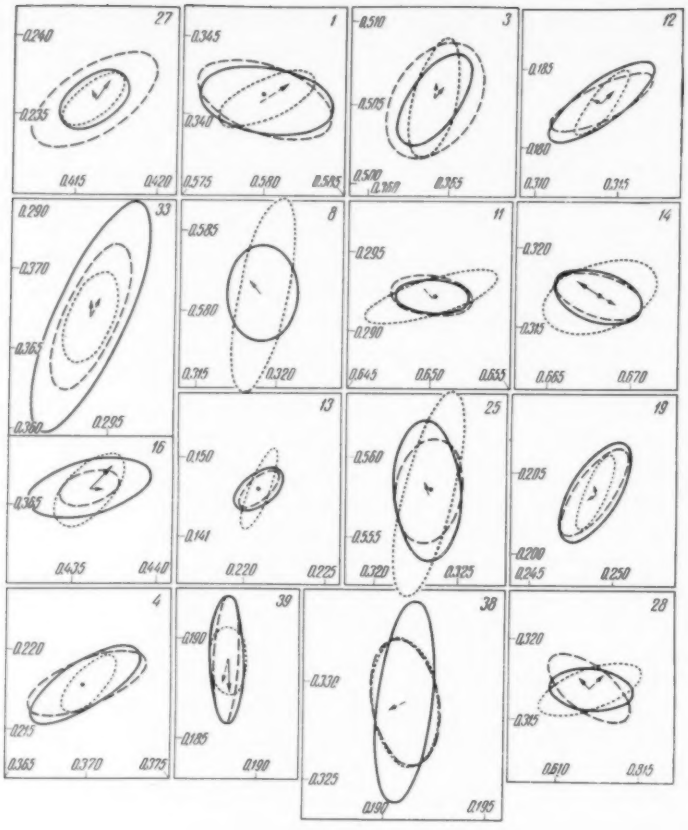

FIG. 2.

which may have enabled him to avoid the difficulties arising from our not having given full details of our co-ordinate system in [2]; but these relationships contain a definite arbitrary element, in particular Ingle and Rudick's expression [8] for describing combinations of colour sensitivity and brightness sensitivity. Might not different results be obtained from our data by starting from other assumptions ?

We can evaluate MacAdam's operations on our data by comparing Fig. 5 from his paper [1], where the results are shown graphically* with Fig. 15 from MacAdam and Brown's paper [5]. Fig. 16 of [5] shows sections of the threshold ellipses for W.R.B.† in the same equal-luminosity plane to which MacAdam transferred our ellipses (Fig. 4).

This comparison shows quite convincingly that there are observers (similar to W.R.B.) whose threshold ellipses in the colour region we used show comparatively small changes in size very similar to those found in our experiments, and which in

* This figure does not show ellipses (dashed) which MacAdam took from our paper of 1952 (with V. P. Solov'eva).

† The ellipses for W.R.B. which fall outside the colour region we used have been cut out.

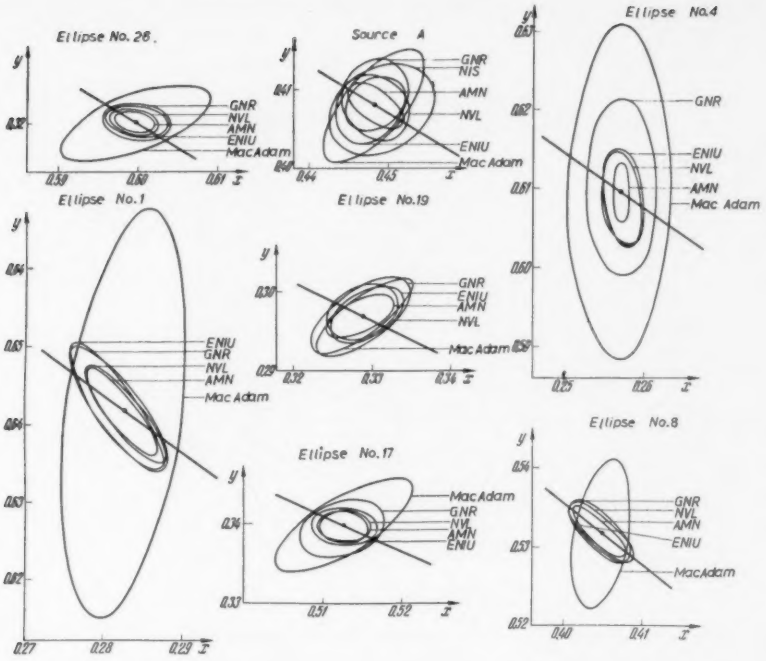

FIG. 3.

any case differ in shape from ours much less than do those of P.G.N. This shows that in P.G.N. the acuteness of colour differentiation along the physiological g-axis is reduced, his threshold ellipses being thus elongated along this direction.

In conclusion we give data from a more complete calibration of our equipment (also carried out in 1951), namely the equations for the colour co-ordinates α, β, γ in

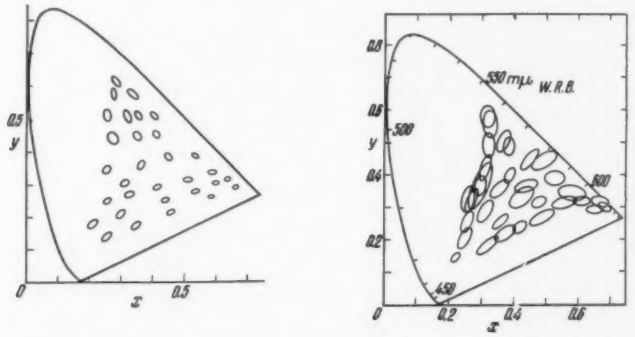

FIG. 4. Threshold ellipses computed from the data for Brown's 12 observers and projected from the equal luminosity plane on to the x–y plane.

the X_0, Y_0, Z_0 system, expressed in terms of the co-ordinates a, b, c, of the colorimeter system R_0, G_0, B_0:

$$\alpha = 1{\cdot}047a + 0{\cdot}182b + 0{\cdot}111c$$
$$\beta = 0{\cdot}453a + 0{\cdot}478b + 0{\cdot}069c$$
$$\gamma = 0{\cdot}015a + 0{\cdot}483b + 0{\cdot}558c.$$

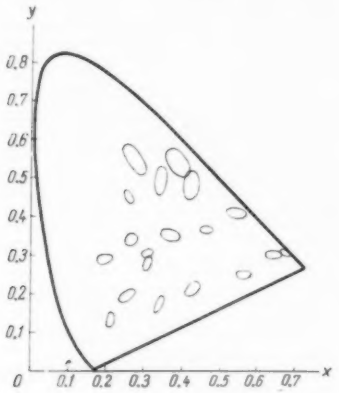

FIG. 5.

Very recently Brown [9] has published fresh threshold ellipsoid determinations for 12 observers and 22 colours. The experimental conditions differed somewhat from the previous ones, and particularly from ours. But all the same it is of interest to compare these data with those discussed above, the more so since they are all presented in the x–y form and refer to the equal-luminosity plane (enlarged by a factor 3, like the threshold ellipses, except for two ellipses falling outside the region of our figure). This figure (Fig. 5) once again shows the variety in ellipse sizes; it is here not so large as MacAdam is forced to assert.

Translated by J. E. S. BRADLEY

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CHANGES IN CO-ORDINATED STRUCTURE OF HUMAN GAIT AT HIGH ALTITUDES*

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(*Received* 27 *May* 1957)

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THIS study of the co-ordinated structure of gait in the mountains was made by the author in 1947 on the approaches to the summit of Mount Kazbek. The gait of three persons was studied (that of two qualified mountaineers and of one novice) walking under a load, on level ground and on slopes of varying steepness, at varying heights above sea level. The cyclographic [1] method of study was used. But since the records were made in the absence of electric current, this meant that special mechanical apparatus had to be designed and constructed, which did, however, serve to execute all the experiments with passable accuracy. In view of the susceptibility of the method to distortion owing to foreshortening, etc., only those data are analysed which occur sufficiently often and which show sharply expressed values.

INFLUENCE OF HIGH ALTITUDE ON CO-ORDINATIONAL STRUCTURE OF GAIT

In order to determine how conditions at high altitudes affect the co-ordinated structure of gait, a comparison should be made of the main kinematic and dynamic indices of the gait, under a load and before fatigue intervenes, of mountain climbers and of a novice, on hard level ground at different altitudes.

If a comparison is made between the left-hand and middle curves in Fig. 1, the difference becomes immediately apparent. The configuration of the waves which are of decisive significance in the act of walking [2, 3] has changed. Their value

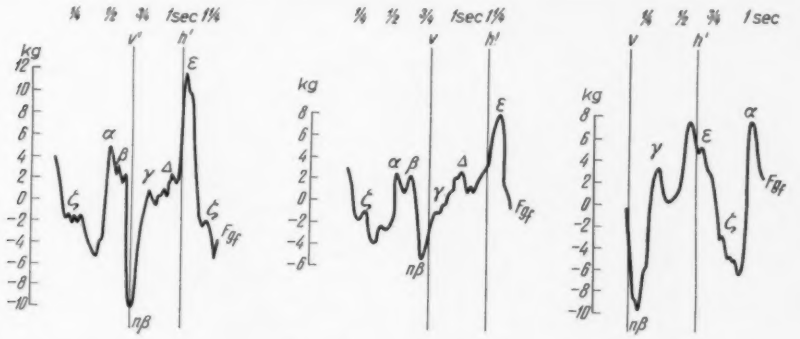

FIG. 1. Changes in configuration of the longitudinal component of the efforts at the centre of gravity of the hip in a trained climber during gait at different heights. Author's experiments. I—gait at height 1750 m, without fatigue; II—the same, at height 3660 m; III—the same at height 3660 m, but with fatigue.

* Biofizika **2:** 5, 642-648, 1957 [Reprint Order No. BIO 85].

TABLE 1. KINEMATIC INDICES OF GAIT AT DIFFERENT ALTITUDES

Indices	Trained climbers				Novice	
	K.		N.		M.	
	1750 m	3660 m	1750 m	3660 m	1750 m	3660 m
Tempo (paces per min)	104·6	106·0	108·0	104·6	89·0	95·0
Length of double stride (in cm)	140·0	140·5	144·2	145·0	155·0	148·4
Duration of double stride (sec)	1·15	1·13	1·02	1·09	1·32	1·24
Speed (cm/sec)	160·0	149·0	166·0	144·0	135·0	142·0
Minimal knee angle (°)	107·0	121·0	103·0	117·0	97·0	115·0

Note: At altitude 3660 m barometric pressure was 494·3 mm.

has also changed, Let us look, however, at indices that can be quantitatively measured.

In Table 1 are given the kinematic indices. This table shows that the trained climbers, although tempo and length of pace had not been set beforehand, kept these parameters with amazing lack of variation, walking all the time at their habitual, steady pace, not lengthening or shortening their stride. The novice behaved otherwise: in his case we see he has no one steady pace. But this table shows something else. If we compare the minimal knee angles (i.e. value of greatest bend of leg at knee) of our subjects when walking at different heights, we can see at once that this angle increases in gait at 3660 m. So, in walking at this height the leg bends less at the knee, a climber's movements become more "economical". Also, their speed diminishes perceptibly.

Let us now turn to dynamic indices. These show the value and direction of the total field of force acting upon the various parts of the legs (Table 2, with identifying letters the same as in Fig. 1).

TABLE 2. DYNAMIC INDICES OF GAIT AT DIFFERENT ALTITUDES (IN KG)

Efforts at centre of gravity of hip (kg)	Trained climbers						Novice		
	K.			N.			M.		
	1750 m	3660 m		1750 m	3660 m		1750 m	3660 m	
		Untired	Tired		Untired	Tired		Untired	Tired
Effort of front impulse, $n\beta$	—8·9	—5·0	—7·5	—8·7	—5·3	—8·2	—6·3	—6·9	—6·8
Flexing efforts of back impulse, γ	9·5	8·3	10·4	11·3	7·6	7·3	5·0	6·7	8·3
Reactive effort occurring after unbending of leg at knee-joint (in carry-over phase), α	6·0	3·9	6·5	4·5	2·7	7·2	4·2	4·3	8·1

If it is borne in mind that the efforts taking place at these points reflect to some extent the activity of the central mechanisms which participate in the act of walking, it is easily seen that the trained climbers, retaining a constant length of pace and tempo of movement, achieve a considerable economy of effort. The decrease in the amount of the flexing effort of the back impulse quite clearly bespeaks the economical nature of their gait. The decrease in the amount of the front impulse, of the braking effort directed backwards against the subject's advance, and therefore having a negative value, is evidence of a general decrease in the force of the dynamic impulses.

But the question of the de-automatization of gait is in the last resort decided by the amount of the reactive* efforts, their values showing decisively whether or not a movement is taking place in dynamically stable form. If this is so, then a decrease in the amount of the leading impulses should be followed by a decrease in the reactive efforts [3]. For our subjects we have a completely clear picture. The trained climbers, accustomed to high mountain conditions, decreased the values of their reactive efforts, both doing so simultaneously, while the novice did not do so. At the same time we do not see in his case any reduction in the basic, leading, efforts; on the contrary, there is some increase in these. This fact points to a certain de-automatization of gait in the novice, under the influence of high mountain conditions, primarily of course rarefication of the air, which is easily seen to be connected with a disturbance of the processes of excitation and inhibition in the central nervous system owing to hypoxia.

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Now let us consider the matter of changes in the above-mentioned indices under the influence of fatigue. In order to check the action of fatigue on de-automatization of gait, in the experiments made at 3660 m we made a control record of gait in our subjects after each had first run 200 m with a load on his back.

It can be seen from Fig. 1 and Table 2 that fatigue heightens the de-automatization that had already set in. The reactive effort (α) increases sharply, which indicates a loss of dynamic stability of pace; the flexing effort decreases (or increases); and the effort of the front impulse grows. All this is evidence of the appearance of the heavy tread down of the foot on to the point of support, so typical of a tired man. It is interesting to note that these phenomena are more sharply expressed in the novice than in the trained climbers.

The above facts lead us to the conclusion that instruction and training in the technique of movement under high mountain conditions causes the indices of co-ordinated structure of gait to undergo definite changes. These are expressed as economy of the basic power components, indicating the working out of an economical pace. When no such changes have been induced, some disturbance of normal automatism of gait is observed at heights of about 4000 m above sea level, even without fatigue intervening.

CO-ORDINATED STRUCTURE OF GAIT ON SLOPES

In the course of the study of the co-ordinated structure of gait in the mountains, a special investigation was devoted to gait on slopes, carrying a load, and without the use of aids such as crampons, spikes, etc.; the object being to shed light on general

* "Reactive" being the word used to describe an effort which arises in the part as the result of the movement next to it.

questions of changes taking place in the co-ordinated structure of gait at different angles of the supporting surface, and at the same time to elucidate the technique of movement on slopes.

This study was made at heights of 1750 and 3660 m. Gait on hard going was observed. The subjects carried loads of 16 kg on their backs, and wore the outfit usual for a mountain climber, with nailed boots.

Co-ordinational structure of gait on an ascending slope

Evidence of the fact that gait on an ascending slope differs considerably in its speed indices from gait on level ground is provided by Table 3, which gives data for K., an experienced mountain climber.

It can be seen that while ascending a slope, the speed of movement and the length of pace decrease noticeably. The length of time for which the foot rests on the point of support increases, and the duration of the carry-over lessens, the duration of the dual-support phase being lengthened as a result. These changes are far from being due to mere chance. They arise out of those changes in the biodynamics of taking a step which are produced by the presence of the slope and by the necessity of placing the foot on a sloping surface.

TABLE 3. KINEMATIC INDICES OF GAIT ON SLOPES

Indices	Gait on level ground	Gait on up-slope		Gait on down-slope	
		15°	30°	15°	30°
Speed (cm/sec)	150	138	78	96	84
Tempo (of single steps) (steps/min)	104	104	100	111	116
Duration of support interval (sec)	0·642	0·667	0·700	0·400	0·333
Duration of carry-over interval (sec)	0·512	0·483	0·500	0·608	0·687
Length of double step (cm)	140	108	68	88	95

Let us start our analysis of the movements made at the point when the leg, having completed the back impulse, starts the carry-over interval (Fig. 2). All the movements of this interval differ considerably when walking on a slope from those made when walking on level ground. On the latter there is nothing to hinder the leg from unbending fully at the knee and hip joints and making a front impulse (at the end of the interval) of corresponding magnitude. In walking on an up-slope, however, the slope does not allow the leg to unbend fully, as a result of which all the straightening efforts (wave $n\zeta$) are considerably weakened, and all the flexing efforts preceding them are reduced in consequence (see Table 4, wave ζ).

In walking on an up-slope the beginning of the carry-over interval proceeds more feebly and passively than in walking on level ground. At the end of it one very typical movement is observed. In going up a slope one must place the foot at the necessary height. If the leg is left to perform only its flexing and unbending efforts, the step will be too short. So climbers who have been trained to walk up mountains give an additional impulse upwards at the end of the carry-over interval, and only after that place their foot on the point of support.

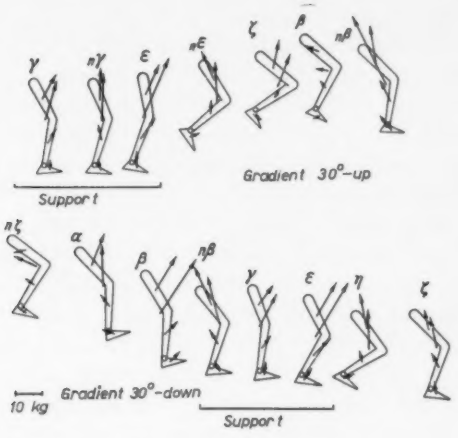

FIG. 2. Positions of parts of right leg of climber, and efforts at their centres of gravity (indicated by arrows) during gait on up-slope and down-slope of 30°. Key to values shown by arrows given below. Author's experiments.

The carry-over interval ends in the front impulse. In walking up a slope, as Table 4 (wave nβ) shows, this impulse is decreased, which is evidence of a dynamic weakening of the carry-over interval. The support interval is much more significant than the carry-over. In walking on the level the leg that is in support must, absorbing the force of the front impulse, push the body forward; on an up-slope it must also lift the walker's body upwards. The impulse becomes an active one, and the degree of its activity is a decisive factor for the tempo of movement (Table 4, increase in value of wave Δ). Also, the back impulse is considerably extended in time and takes in almost the whole of the carry-over interval (Fig. 2).

The movements of the leg during the support interval can generally be reduced

TABLE 4. PRINCIPAL DYNAMIC EFFORTS IN GENERAL CENTRE OF GRAVITY OF LEG (IN KG) IN GAIT ON UPWARD AND DOWNWARD SLOPES

Efforts	Gait on level	Up-slope		Down-slope	
		15°	30°	15°	30°
Flexing efforts of start of carry-over interval, ξ	16·8	9·4	9·7	9·8	13·2
Unbending efforts of end of carry-over interval, ζ	—4·9	—3·9	—0·6	—5·1	—11·6
Front impulse, nβ	—13·4	—8·8	—8·0	—9·3	—11·6
Start of back impulse, Δ	2·5	8·2	—6·5	4·5	6·4
End of back impulse, γ	21·4	21·3	27·5	23·2	24·2
Maximum knee angle (°)	180	170	147	175	149
Minimum knee angle (°)	107	99	69	109	83

Note: A negative effort is directed against the line of advance (backwards), and a positive one with that line (forwards).

to the following. Support starts with the absorption of the front impulse. The moment at which this occurs corresponds to the end of the support interval of the other leg and, consequently, to the double support phase. Further, after the other leg has started its carry-over interval, the support leg begins the back impulse. From its very first instants this follows the development of the movements of the transfer leg and reaches its maximum when that leg gives the additional upward impulse described above. The peculiarity of the back impulse lies in the fact that it is given with the shin almost motionless (it "gives" forward a little only at the end of the support period) and is a movement which, as it were, draws the whole body of the walker forward and upward, after the "transfer leg" and turning in relation to the knee-joint. It is the movement which ensures the lift of the body to a given height. We called this movement "pulling up on the support foot", and consider it to be a decisive factor in the rhythmic and motor characteristics of gait on an up-slope.

The completion of the support interval of one leg (the "pulling-up") coincides with the end of the carry-over interval of the other leg, which now treads down on to the point of support. The phase of double support begins, after which both legs continue their movements but with the roles reversed: the leg that has been in support comes away from the ground, while the other performs the movements of the support interval.

Co-ordinational structure of gait on a down-slope

As Table 3 and Fig. 2 show, gait on a down-slope also differs considerably from gait on level ground. As the gradient increases, the speed of advance of the walker diminishes, while the tempo of gait increases. The length of time spent by the leg in support shortens, and the duration of the carry-over interval lengthens. The result of this is that the phase of double support is reduced almost to nothing. The cause of these changes lies in the fact that the back impulse—the basic effort in walking up a slope or on the level—loses its primary importance, which is taken over by the front impulse—the braking effort by which the walker contends with the force of gravity. The phenomenon is familiar to anyone who has ever walked downhill.

Let us consider the dynamics of gait on a down-slope in the same order as we followed for those of gait on an up-slope. We will start with the carry-over interval. In walking downhill it is very important to unbend the leg and throw it forward as much as possible. The unbending efforts of the carry-over interval and the flexing efforts (waves ξ and ζ) are always increased accordingly in walking downhill (Table 4). In consequence the carry-over interval assumes the following form: after a phase of double support considerably shortened in time (or more precisely almost absent), the leg is thrown forward and downward by a much weakened back impulse, and bends at the knee and hip. Then come very active unbending efforts, by which the leg unbends to a certain degree and then in that position prepares to support. These efforts, however, are not enough. If the leg is left to the effect of the unbending efforts alone, the front impulse will be too sharp. So, before the leg treads on to the point of support an additional braking is observed (increase of wave $n\beta$), the object of which is to soften the impact of the foot on the ground.

The front impulse ends the carry-over interval and starts the support interval. In walking downhill, as we have already mentioned, it is not the back but the front

impulse which plays the leading part, and this grows more and more important the steeper the slope. As soon as the support leg takes the weight of the body and the other leg has initiated the movement of the carry-over interval, the active intervention of the support leg begins: it bends at the knee-joint, and the walker makes a kind of curtseying movement upon it. The object of this is to make things easier for the movements of the carry-over leg, which at this moment is completing its unbending efforts and preparing to go into support. The "curtsey movement" enables it to lengthen the trajectory of its movement a little and to make the step of a more rational length.

The "curtsey" ends at the moment when the carry-over leg touches support, after which the legs, exchanging roles, continue making movements in the order described.

CONCLUSIONS

(1) Normal gait in the mountains shows a disturbance of a number of elements in its co-ordinational structure at a height of about 3500 m. Fatigue intensifies this disturbance.

(2) Gait on sufficiently steep upward and downward slopes shows a sharp change in its normal automatism. New elements appear and old ones disappear in its dynamic components, though a number of these remain the same.

Translated by R. KISCH

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THE IMPORTANCE OF INTENSITY AND SPECTRAL COMPOSITION OF LIGHT FOR PIGMENT FORMATION BY PLANTS*

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(*Received* 22 *March* 1957)

THERE is a large literature devoted to the study of the synthesis and accumulation of pigments in plants. Since the formation of the majority of the pigments is a photochemical process, the fundamental problem with which investigators have been concerned has been the study of the role of various parts of the solar spectrum in pigment synthesis. At the same time a study has been made of the correspondence between

* Biofizika **2**: 6, 649–660, 1957 [Reprint Order No. BIO 86].

the action spectra for pigment formation and the absorption spectra of the precursors of these pigments, and also of the absorption spectra of the pigments themselves. The Russian scientists Timiryazev [1, 2] and Monteverde and Lyubimenko [3] did much towards solving this problem; they considered that the synthesis of the green pigment, chlorophyll, is accomplished best of all in the red region of the spectrum, i.e. with those radiations which are most strongly absorbed by chlorophyll. Experimenting with various light-filters and thus separating out individual spectral regions, Monteverde and Lyubimenko [3] established that red and red–orange rays are more effective than blue for the photochemical process of chlorophyll synthesis.

A series of studies can be brought forward which confirm the higher activity of red and red–orange rays in the "greening" of plants. One can quote such investigators as Sayre [4], Rudolph [5], Seybold and Egle [6], Simonis [7], Strott [8] Went [9], Rubin and Chernavina [10].

Went [9] and Rudolph [5] established that chlorophyll formation takes place at the same speed in red and yellow–green light and slower in blue. Lease and Tottingham [11] established that the greatest amount of chlorophyll is formed on adding blue–violet light to the red.

In Wiesner's work [12] we meet an indication that, with a high level of radiation, a more intensive accumulation of chlorophyll occurs in blue light than in yellow, but with weaker illumination "greening" goes faster behind a yellow screen than behind green, red or blue, in this order. Of course, in these experiments the luminous intensity was not kept constant and, clearly, the radiation was too weak in the measurements with the red and blue filters.

Frank [13], working with etiolated oat seedlings, used 16 light-filters which passed narrow spectral bands and which were made equivalent with respect to the amount of radiant energy they passed. He demonstrated the existence of two optimal wavelengths for chlorophyll synthesis, one in the red region at 645 mμ and the other in the blue at 445 mμ; and two sub-optimal wavelengths, at 575 mμ and 545 mμ.

Thus, according to Frank's data, the action spectrum for chlorophyll formation coincides with the absorption spectrum of the assumed precursor of chlorophyll—protochlorophyll.

In his account of the action of light on plants, Kleshnin [14] cited his own experimental data, obtained together with Osipova and Timofeeva [15], that the pigment content of lettuce plants was higher in red light. It should be noted that Kleshnin's data illustrate the level of pigment accumulation in different states and developmental stages of the plants, rather than the effect of different regions of the spectrum on pigment formation. In blue light the experimental lettuce plants were in the rosette stage and were rapidly growing vegetatively, but in red the plants passed over to fruit-bearing and had a leafy, flower-bearing, shoot.

One can also quote the work of Rubin and Chernavina [10] who studied pigment accumulation in wheat leaves. They employed coloured boxes with coloured cellophane and with gauze covers. Quantitative data on the radiation intensity were not given in their paper, but the use of gauze covers as light flux equalizers allowed them to assume that the radiation intensity in the chambers was low in the experiments. The authors concluded that red–orange illumination creates the most favourable conditions for chlorophyll synthesis in winter and spring wheat. However, very recently

Chernavina [16], in work which is undoubtedly a direct continuation of the work of Rubin and Chernavina [10], comes to the opposite conclusion, which she formulates thus: "blue rays, as compared with red, provide a more intensive accumulation of both green and yellow pigments during the first stages of their formation".

How can these two points of view be accommodated if the author makes no reservations about the diametrically opposite results obtained in the previous paper?

It is obvious that these contradictory conclusions are a consequence of neglecting to calculate the radiation level, distributed over the spectrum, in the various series of studies. As we shall see later, our data prove that the radiation intensity is very important when studying the effect of different parts of the spectrum on pigment formation.

As for the associates of chlorophyll—the yellow pigments, carotin and xanthophyll—there are very few facts about the influence of light of various wavelengths on their synthesis and accumulation in the plastids. It is well known that carotenoids are formed in the dark and that, for their synthesis, they do not need radiant energy of external origin; all the same Giubbenet [17] noted a considerable accumulation of carotenoids in etiolated seedlings exposed to light. Rudolph [5] and Simonis [7] pointed out the faster formation of carotenoids in blue light, which coincides with the absorption spectrum of the carotenoids themselves. At the same time, according to Strott's data [8], carotenoids, like chlorophyll, are formed faster in the red region of the spectrum.

A higher carotenoid content in lettuce leaves growing in red light was also noted by Kleshnin, Osipova and Timofeeva [15]. However, as pointed out above, the authors themselves suggest that these results are chiefly due to differences in the plants' developmental stages.

In Rabinovich's vast account of photosynthesis [18], only brief indications are given that the synthesis of chlorophyll and carotenoids is determined, on the one hand by the position of the absorption band of their precursors, and on the other by the result of "autosensitization" of the pigments themselves. Therefore, the action spectrum of the synthesis of chlorophyll and carotenoids might be determined by their absorption spectra.

It should be pointed out that, when studying biosynthesis and the conditions of pigment accumulation, various authors have experimented with the most diverse material, from single-celled algae and etiolated seedlings to green plants of various ages. It is also necessary to note the use of very diverse conditions of illumination, from sunlight, filtered by various light-filters, to artificial light sources which have different spectral characteristics. The existing divergences in the estimates of the influence of different spectral regions on pigment formation can be related to this diversity of material, irradiation techniques and methods of equalizing and measuring radiant energy. Thus, in spite of the large amount of work, there is still no well-defined solution of the problem of the position of the maximum in the action spectrum of the synthesis and accumulation of pigments in plants.

Proceeding now to our study of the laws of the energetics of the green plant under various conditions of illumination, we began our investigations with a study of the optical properties of the green leaf, the first stage in which is the present work on the features of pigment accumulation in green plants on irradiating them with light of various spectral regions and at various levels of illumination.

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Coloured luminescent lamps served as sources for obtaining separated parts of the spectrum (blue and red) and white luminescent lamps, daylight type DS, were taken as controls. The lamps were mounted in frames in such a way that a continuous illuminating ceiling was created. The individual chambers with coloured light were isolated from each other by double screens, the inside ones white and the outside of opaque material. The spectral characteristics of the luminescent lamps are given in Fig. 1.

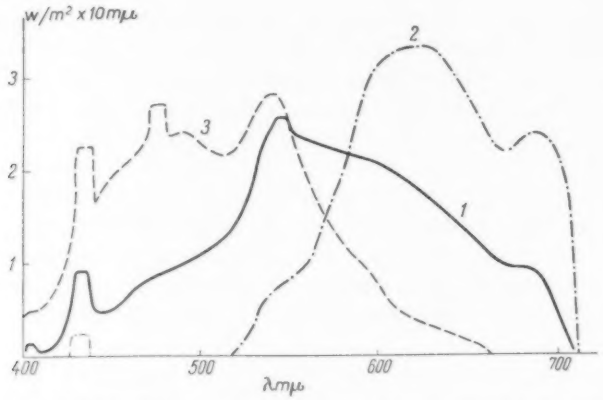

FIG. 1. Distribution of energy in the spectrum of the coloured luminescent lamps: (1)—white, DS; (2)—red; (3)—blue. Ordinate: spectral intensity, W/m² per 10 mμ; abscissa: wavelength λ, mμ.

From this figure it can be seen that the blue luminescent lamps radiate in the region 400–660 mμ, with a maximum between 460 and 540 mμ; the red lamps cover the region 520–710 mμ with a maximum between 620 and 680 mμ; the lamps DS (white) radiate the whole visible spectrum, i.e. in the region 400–710 mμ with a maximum at 550 mμ.

The spectral characteristics of the coloured luminescent lamps were obtained by a universal monochromator with the help of a selenium photo-element with a known spectral characteristic.

Equalization of the radiation intensity under the different coloured luminescent lamps was carried out at the level of the upper leaves by means of a Yanishevskii pyranometer, which possesses a non-selective sensitivity in the light region which interests us (400–720 mμ). The absence of near infra-red radiation in the given source was verified by the same apparatus with light filters IKS-1 and KS-19 placed on it. Air temperature and the other conditions under the lamps in the various chambers were the same. The radiation intensity in different experiments with the coloured lamps varied from 18 to 35, and under the DS lamps from 40 to 80 W/m² (18,000–80,000 erg/cm² sec) of radiation physiologically beneficial for plants.

In order to study the influence of narrower bands of the spectrum, coloured light chambers were used; a diagram of the chambers is given in Fig. 2. Reflector lamps of 300 and 500 W (ZN-7 and ZN-8) served as radiation sources. All the lamps were immersed in running water in a common vessel with a glass bottom. The water

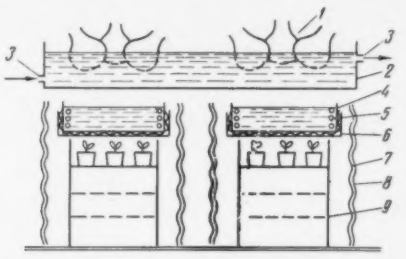

FIG. 2. Diagram of construction of coloured light chambers. (1)—reflector lamps; (2)—common running-water filter with glass bottom; (3)—water inflow and outflow; (4)—stationary liquid colour filter; (5)—coil containing running water for cooling the liquid filter; (6)—cellophane coloured light-filter; (7)—white screens; (8)—black screens; (9)—stand on which the plants are placed.

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cooled the lamp bulbs and partially absorbed the infra-red radiation. Underneath the common running-water filter, coloured light chambers with various liquid and film light-filters were placed; the chambers were isolated from each other by opaque screens. The liquid light-filters were in crystallizing dishes, to the bottom of which, on the outside, a coloured cellophane filter was fixed. In order to prevent overheating of the liquid light-filters—because of absorption of infra-red radiation—coils containing running cold water were immersed in the solutions in the crystallizing dishes. By regulating the speed of the current of water through the coils, we kept the same temperature in the chambers.

As light-filters we used cellophane, coloured in dye solutions, and liquid light-filters. In the red chamber this was 2 % $CuSO_4$ solution in a layer of 4 cm, for absorption of the infra-red radiation, and two layers of cellophane dyed with safranin (10 g in 1 l. of water).

In the blue chamber the filter was a 25 % $CuSO_4$ solution in a layer of 5 cm, and one layer of cellophane dyed with methylene blue (10 g in 1 l. of water).

The spectral characteristics of the light-filters employed (film and liquid) were measured by universal monochromator UM-2. The spectral intensity of the radiation in the chambers was calculated by the formula:

$$I_\lambda = \tau_\lambda' \cdot \tau_\lambda'' \cdot I_\lambda^\circ$$

where τ_λ', τ_λ'' are the transmission coefficients of the light-filters, and I_λ° is the spectral intensity of the radiation from the incandescent lamps.

The spectral characteristics of the radiation in the coloured light chambers are given in Fig. 3. From this diagram it can be seen that in the blue chamber the radiant flux covers the region 400–550 mμ with a maximum at 450–500 mμ; in the red chamber it covers 600–740 mμ with a maximum at 660 mμ. The temperature and other conditions in the chambers were identical. The radiation intensity in different experiments varied from 15 to 18 W/m² (15,000–18,000 erg/cm²sec).

Carotenoids and chlorophyll were determined by Godnev's method [19]. The extinction of the pigment extracts was measured on a photoelectric colorimeter (FEK-M).

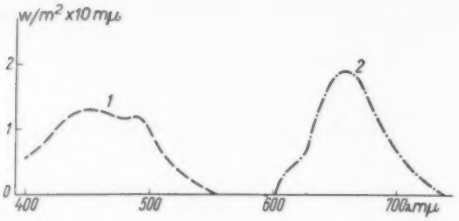

Fig. 3. Spectral characteristics of the illumination in the coloured light chambers with liquid light-filters. (1)—blue chamber; (2)—red chamber. Ordinate: spectral intensity W/m^2 per 10 mμ; abscissa: wavelength λ, mμ.

In some parallel experiments, on plants grown under the same conditions, the carbohydrates were determined by the micro-method of Hagedorn and Jensen.

The experimental plants were diverse in their systematic and morphological features: cereals (winter and spring wheat, maize), cucumber, horse bean, radish and lettuce. The age of the plant and the stage of the leaves were recorded when the accumulation of the pigments was determined.

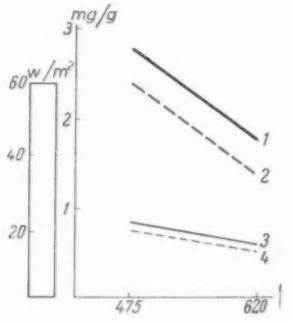

Fig. 4. Pigment content in the leaves of 33 day old maize grown under coloured luminescent lamps. Chlorophyll: (1)—fifth leaf; (2)—third leaf. Carotenoids: (3)—fifth leaf; (4)—third leaf. Ordinates: illumination, W/m^2, and pigment content, mg/g fresh weight. Abscissa: wavelength λ, mμ.

In Fig. 4 and Table 1 the results of some experiments are given on the determination of the amounts of pigments (chlorophyll and carotenoids) in the leaves of cucumbers and horse beans which were grown under red and blue luminescent lamps at the optimal level of illumination (40–60 W/m^2).

The data of these experiments show a considerably greater accumulation of pigments in plants grown in blue light than in plants grown in red light.

In order to study the influence of pigment formation of narrower spectral regions, parallel experiments were carried out in the coloured light chambers with light-filters. However, in view of the impossibility of obtaining in the coloured chamber as high a radiant flux as we worked with under the coloured luminescent lamps, our experiments with a narrower spectrum of radiation were done at a much lower intensity (15–18 W/m^2). At the same time we encountered another law concerning the

dependence of pigment accumulation on the action on the plant of different spectral regions. We quote the experimental results for two kinds of wheat: winter, Lutescens 329; and spring, Lutescens 062.

TABLE 1. PIGMENT CONTENT (mg/g WET WEIGHT) in 7 DAY SEEDLINGS OF WHEAT GROWN IN COLOURED LIGHT CHAMBERS AT AN INTENSITY OF ILLUMINATION OF 18 W/m²

Kind	Chlorophyll		Carotenoids	
	Blue	Red	Blue	Red
Winter wheat 329	1·06	1·50	0·40	0·55
Spring wheat 062	1·03	1·69	0·46	0·73

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As can be seen from Table 1, with a low radiation intensity the greatest accumulation of pigments was observed when the plant was exposed to red light, and the smallest accumulation when acted upon by blue light.

Such an inconsistency in the results of the two different series of experiments made us consider the possibility of a specific influence due to the different characters of the irradiating light: in the colour chambers with light-filters and in the arrangements with coloured luminescent lamps.

In order to make the results more precise, experiments were carried out on the same kinds of wheat as in the above experiment. The plants were grown under the following conditions:

(1) in the colour chambers at a radiation level of 16–18 W/m²;
(2) in the coloured luminescent lamp arrangement:
  (*a*) at a radiation level of 18–20 W/m²,
  (*b*) at a radiation level of 35 W/m².

The experiment lasted 12 days and the pigments were determined on three occasions during the plants' growth. The data on winter wheat are given in Fig. 5.

As can be seen from the course of the curves of pigment content in winter wheat, we obtained confirmation of our earlier results, i.e. at a lower level of radiation the

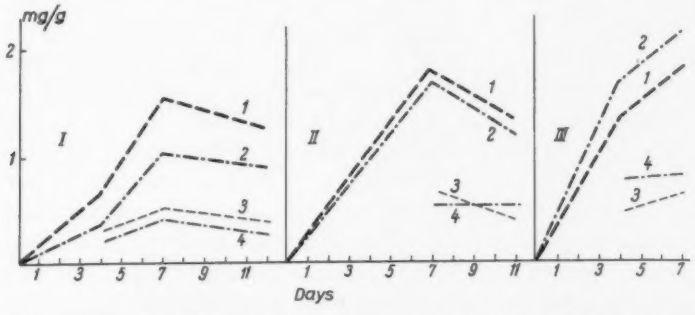

FIG. 5. Pigment content in leaves of winter wheat, grown: I—in the colour chambers with an illumination of 18 W/m²; II—under the coloured luminescent lamps at 18 W/m²; III—under the coloured luminescent lamps at 34 Wm/². Chlorophyll: (1)—red; (2)—blue. Carotenoids: (3)—red; (4)—blue. Ordinates: pigment content in mg/g fresh weight. Abscissae: number of days from germination.

greater pigment accumulation is observed in leaves of the plants in the red chambers. However, under the luminescent lamps at the same low radiation level, the difference in the pigment contents of plants from the red and blue chambers was lower than in plants in the colour chambers. This might be connected with the wider radiation spectrum of the luminescent lamps.

If we compare the data on the pigment content in the leaves of plants from the colour chambers with those with luminescent lamps at different radiation levels, then our conclusion on the higher level of pigment content in plants grown in the blue chambers at a higher radiation level is well confirmed. A higher pigment content, and not only of chlorophyll but also of carotenoids, is observed at the higher level of illumination in both the red and blue chambers. This can undoubtedly be connected with the presence of a greater amount of initial products for the synthesis of the carbohydrate precursors of chlorophyll. There will undoubtedly be greater amounts in plants grown at a higher light intensity. In his time Palladin [20] pointed out the direct connexion between the presence of carbohydrates in seedlings and the intensity of the greening process. At the present time, since the work of Godnev and Shlyk [21, 22], no doubts remain of the direct, generic, connexion of carbohydrates with one of the initial products of synthesis of the precursors of chlorophyll.

Also, in Frank's interesting work [23], one important property of carbohydrates for pigment accumulation has been established, namely their protective action against decomposition of the pigments in the dark.

From a general comparison of the data of Table 1 and Figs. 4 and 5 on the effect of red and blue light on pigment accumulation in the leaves of various plants, we can note the specificity of the effect of these different regions of the spectrum.

Irradiating plants with red light of small or great intensity does not lead to a considerable increase in pigment content as compared with the amounts formed at low radiation levels. Thus, the impression is created that there is some optimum level of pigment concentration in plant leaves in red light, which is sufficient for the normal photosynthetic requirements of plants which use as a source of radiant energy the red spectral region, which is best absorbed by chlorophyll. This optimum pigment content level is provided in red light at the comparatively low illumination of 15–20 W/m^2.

As for the effect of blue light, a marked increase in pigment content occurs in parallel with increase of intensity of illumination and, as we have already remarked, at high blue-light illumination levels the plants also have a greater pigment accumulation as compared with red light, which is well shown in Fig. 6. Clearly, such differences in rates of synthesis and accumulation of pigments in blue and red light are connected with the fundamental function of the pigments and, in particular, for chlorophyll, with the process of photosynthesis. In order that chlorophyll should perform satisfactorily in blue light, when it absorbs less radiant energy than in the red, the pigment concentration should clearly be higher so that the photosynthetic apparatus can work with the same effectiveness as in red light.

In parallel with the above-described experiments, an investigation was carried out on pigment accumulation in plants grown under DS luminescent lamps at a high radiation intensity (60–80 W/m^2) and with a length of "day" of 14 hr; this was followed by placing the plants under the coloured luminescent lamps at a considerably

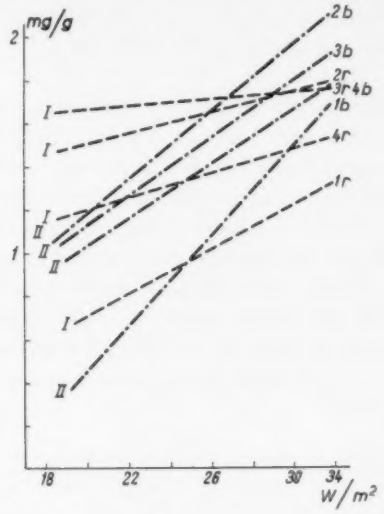

FIG. 6. Chlorophyll content in leaves of winter wheat (1 and 2) and spring wheat (3 and 4), grown in the colour chambers and under the coloured luminescent lamps at various levels of illumination. 1—red (k); II—blue (c). Ordinate: pigment content in mg/g fresh weight; abscissa: illumination in W/m².

lower radiation level than with the DS lamps, 30 W/m², for three days and with a 24 hr photoperiod. Before placing them under the coloured luminescent lamps the plants were kept in the dark where, as a result of the process of respiration, a considerable decrease in the carbohydrate content took place and, at the same time, a decrease in the pigment content was observed. The experimental results on maize are presented in Fig. 7. As can be seen from the figure, the most severe decrease in

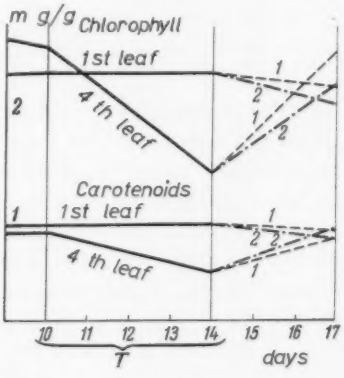

FIG. 7. Pigment content (mg/g fresh weight) in the leaves of maize grown under the DS luminescent lamps at an illumination of 75 W/m², transferred to the dark and then placed under the coloured luminescent lamps at 30 W/m². T—period plants are in darkness. (1)—red; (2)—blue. Abscissa: age of plants, days. Ordinate: pigment content, mg/g fresh weight.

pigment content in the dark is observed in leaves in a state of active growth. At the same time the decrease in pigment content of leaves which have finished growing is very small.

On transferring plants, grown at a high light intensity (80 W/m²) under DS lamps (after a stay in the dark) to the coloured luminescent lamps at a lower radiation level, 30 W/cm² (but for a greater length of illumination, 24 hr daily), pigment formation proceeds more actively in red light, just as in those plants grown at low radiation levels. In blue light resynthesis is observed only in growing leaves; in leaves which have finished their growth the pigment content continues to fall even when the plants are transferred to light. These disturbances in pigment formation, which take place in green leaves in the dark, are not overcome by transferring the plants to blue light.

As a development of our studies on the influence of light of different intensities on pigment synthesis, we performed an experiment on maize according to the scheme set out in Table 2.

Table 2. Scheme of irradiation of three groups of maize plants

Variant	Days: 1–10		10–14		14–17	
	VIII		VIII		VIII	
	Radiation (W/m²)	Length of photoperiod (hr)	Radiation (W/m²)	Length of photoperiod (hr)	Radiation (W/m²)	Length of photoperiod (hr)
I	80	14	Darkness		30	24
II	80	14	Darkness		80	24
III	80	14	80	14	80	24

The results of measurements of the pigments and carbohydrates in all the plant groups of this experiment are set out in Table 3.

The experimental results clearly show that the pigment system of the first leaf, the oldest, is more stable to the effect of darkness than that of the young leaf which is actively growing.

On transfer of the plants from darkness to the DS luminescent lamps at a radiation level of 30 W/m² for 72 hr (Table 3) a noticeable increase in the chlorophyll content was observed only in the young fourth leaf. The chlorophyll content continued to decrease in the first leaf and, even in light, pigment resynthesis was not observed.

On transfer of the plants from darkness to the DS luminescent lamps at a radiation level of 80 W/m² (II in Table 3), the same regularity was observed as at a radiation level of 30 W/m², the only difference being that their pigment content was higher.

On changing over the plants from a 14 hr photoperiod to an uninterrupted 24 hr irradiation at an intensity level of 80 W/m² (III in Table 3) a sharp decrease in the chlorophyll content was observed, especially in the ageing first leaf, and a smaller decrease in the chlorophyll content of the young fourth leaf.

The dynamics of the carotenoid contents as a function of change in light intensity and length of photoperiod are characterized by the same basic regularities which are observed for chlorophyll, except that on changing over the plants to continuous

TABLE 3. EXTRACTS FROM MAIZE PLANTS TREATED ACCORDING TO THE PLAN OF TABLE 2, IN mg/g WET WEIGHT

	Leaf	10/VIII	14/VIII	17/VIII		
		Variants				
		I, II, III	I, II	I	II	III
Pigments:						
Chlorophyll	1	2·30	2·28	1·79	2·01	1·13
	4	2·48	1·36	2·19	2·38	1·93
Carotenoids	1	0·85	0·83	0·73	0·79	0·82
	4	0·82	0·47	0·71	0·88	0·75
Carbohydrates:						
Sugars	1	84·6	63·5	66·0	88·7	111·8
	4	72·3	43·3	57·9	63·5	70·1
Starch	1	1·0	4·1	15·1	50·3	61·0
	4	6·9	0·0	0·0	80·9	79·8
Sum	1	85·6	67·6	81·1	149·0	172·8
	4	79·2	43·3	57·9	144·4	149·9

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irradiation at a high intensity the severe decomposition of the carotenoids, which was noted for chlorophyll, was not observed.

As for the carbohydrate dynamics, the decrease in carbohydrate content (soluble and starch) on keeping the plants in the dark should be noted. The greatest fall in carbohydrates was noted in the younger leaves, which had still not finished growing and which have the more intense respiration. In the plants transferred from darkness to DS lamps at an illumination of 30 W/m², an increase in the first leaf was observed in the carbohydrate content almost to the original level, chiefly at the expense of the soluble sugars, while the chlorophyll content goes on decreasing. In the fourth, young, leaf the amounts of carbohydrates only slightly exceed the dark level, but there is a considerable increase in the pigment level which almost reaches the original value. For plants transferred from the dark to conditions of high illumination and 72 hr continuous irradiation, in the leaves at all stages a large accumulation of carbohydrates is observed which considerably exceeds their content in the original material and in particular there is an intensive synthesis of starch.

The great increase in the amounts of carbohydrates in all stages of plant leaves, which are transferred from a 14 hr day at a high radiation level to a 24 hr continuous illumination, is noteworthy. It is interesting to observe that this takes place in spite of a parallel decrease in chlorophyll content. Thus, for example, the chlorophyll content of the first leaf falls by approximately a factor 2 while its carbohydrate content doubles as compared with the original level. Such an increase in carbohydrate content was also observed in the fourth leaf, on a background of some decrease in chlorophyll content.

On the basis of these experiments it can be remarked that a low illumination level (30 W/m²), after keeping plants grown in an illumination of 80 W/m² in the dark cannot restore both pigment formation and carbohydrate accumulation to the original

level which the plants had before putting them in the dark. On illuminating plants, transferred from the dark, by a powerful radiant flux of 80 W/m², the photosynthetic apparatus works more productively and, in spite of a smaller chlorophyll content, secures a greater pigment accumulation.

Similar results were also obtained on transferring plants from a 14 hr day to a continuous, 24 hr, illumination. Notwithstanding a considerable decrease in the pigment content, their photosynthetic capacity grows sharply and provides a considerable increase in the amounts of photosynthetic products.

CONCLUSIONS

On the basis of the above material the following conclusions can be formed.

(1) Pigment accumulation by plants is determined not only by the spectral composition of the illuminating light, but also depends to a great extent on its intensity. The spectral position of the illumination suitable for maximum pigment accumulation can change with the intensity of the radiation, as our experiments have shown. Our work has also shown that, at a low light intensity, pigment accumulation is more rapid in the red part of the spectrum. This may be connected with the greater accumulation of carbohydrates in red light and this provides better conditions for the synthesis of pigment precursors. At a higher intensity of illumination a greater pigment accumulation in leaves takes place in blue light. This might be connected with changes in the rate of the oxidation-reduction processes in plants due to the action of high intensity blue light [24].

(2) It should be noted that due attention has not been paid in the literature to the factor of light intensity, when considering the action spectrum for pigment accumulation. No references to this are found in the most recent book on pigments [25]. The effect of different spectral regions is very often compared using quite unequal amounts of incident energy, and even when the radiant fluxes are equalized they are usually very low; this possibly explains the often-encountered conclusion that the effect of the red spectral region on the synthesis of pigments, especially chlorophyll, is greater than that of other regions.

(3) The carotenoid content of plants is more constant under the action of light of varying spectral composition and changes less when the intensity is changed.

(4) The pigments of the upper, young, leaves are more labile, and are destroyed in the dark to a considerably greater extent, than are the pigments of old leaves. Pigment resynthesis, on transfer of plants from dark to light, takes place more intensively in young leaves than in old.

(5) The young upper leaves of plants contain a greater quantity of pigments than the older leaves of the lower levels. When leaves age, their pigment content decreases.

(6) The reaction of a plant to change of one and the same factor can be different depending on the state of the plant and its previous history. This circumstance, unfortunately, is not always taken into account in studies of the complex internal laws of the living organism, for individual processes are considered irrespective of external influences and the general condition of the organism as a whole.

(7) When studying the optical properties of leaves and the energy balance of plants it is necessary to pay attention to the spectral composition and intensity of the

incident light, and it is especially important to take into account the influence of the light conditions to which the plant was subject before the beginning of the experiment.

Translated by J. DAINTY

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CHANGE OF ADENOSINE TRIPHOSPHORIC ACID CONTENT IN FROG MUSCLE UNDER THE INFLUENCE OF DIFFERENT LOADS*

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(*Received* 24 *October* 1956)

IN a whole series of studies on the influence of the load on the functional properties and metabolism of muscle [1–7] we have demonstrated a dual effect depending on the magnitude of the load. Up to some definite magnitude, which is characteristic for each muscle, the load evokes increases in the muscle's efficiency, in its resistance to stimulation (increase of threshold), in the latent period of stimulation, in electrical potential, in "resting" heat production and in the rate of its "weakening" process.

If the load is further increased, these properties of the muscle change in the opposite direction: the muscle's efficiency begins to fall, its excitability begins to increase again, the electrical potential decreases, the latent period begins to decrease and the rate of the process of "weakening" of the muscle falls. For the frog gastrocnemius muscle this limiting load, at which reverse changes in its functional properties begin, was established to be, on the average, about 200 g depending on the size of the muscle. Geiman [8, 9] has demonstrated changes in ammonia production in the resting frog muscle under the influence of various loads and at the same time he established the dependence of ammonia formation on the magnitude of the load. In normal frog gastrocnemius muscles loads of up to 100 g evoked no changes in ammonia content. In 5 hr a load of 200 g caused an increase in ammonia production of only 25 per cent, on the average. But in the same interval of time a load of 400 g evoked an increase in ammonia production of 146 per cent.

Bauer and Borzdyko [10] demonstrated an increase in creatine phosphate decomposition in frog muscle, increasing with increasing load. In Hill's laboratory Feng [11, 12] detected a two-phase effect of load on the "resting" heat production of a muscle and on its oxidation processes. Up to a certain value, the load evokes an increase in the "resting" heat production and oxidation processes; on further increasing the load both the "resting" heat production and the oxidation processes begin to fall.

All these facts show that the increase in the muscle's functional properties, evoked by a load up to a certain value, depends on an increase in the intensity of its resting metabolism; and that the decrease in the muscle's functional properties, which is observed on further increasing the load, is connected with disturbance of the metabolism caused by the effect of the passage of the load's value beyond the limits of accommodation of the tissue. In the light of these facts there is special interest in a study of muscle adenosine triphosphoric acid changes with loads in this

* Biofizika **2**: 6, 661–664, 1957 [Reprint Order No. BIO 87].

range, for adenosine triphosphoric acid and its conversions are the most significant links in the muscle's energy balance, determining its normal resting and functional condition.

METHODS

The experiments were on isolated frog gastrocnemius muscles. Both pairs of muscles were suspended in a moist chamber—one without a load and the other with —for a definite time; after this the adenosine triphosphoric acid of both muscles was determined by the standard method described in Ferdman's book "Metabolism of Phosphorus Compounds". The amount of phosphorus was determined colorimetrically. The loads we applied were in the same range as in our previous work, i.e. from 50 to 400 g.

EXPERIMENTAL DATA

The experimental results obtained are given in Table 1, where the value of the applied load, its time of action and the amount in mg of adenosine triphosphoric acid in both muscles, experimental and control, are shown. These results are in full agreement with those obtained earlier in the sense that they show the same trend in two directions of the conversion of adenosine triphosphoric acid in muscle, depending on the magnitude of the load.

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TABLE 1. CHANGES IN MUSCLE ADENOSINE TRIPHOSPHORIC ACID CONTENT UNDER THE INFLUENCE OF VARIOUS LOADS

Date of experiment	Load (g)	Time for which load is suspended (hr)	ATP content in muscle (mg)	
			Experimental	Control
11 Oct. 1949	50	1	1·96	1·24
24 Dec. 1949	50	1	2·28	1·08
24 Dec. 1949	50	1	1·44	1·20
12 Oct. 1949	100	1	3·10	1·40
27 Oct. 1949	100	1	1·68	1·20
23 Dec. 1949	100	1	1·44	0·96
23 Dec. 1949	100	1	1·56	0·84
27 Oct. 1949	200	1	1·80	1·80
26 Oct. 1949	200	1·5	1·20	1·20
27 Dec. 1949	200	1	0·84	0·84
27 Dec. 1949	200	1	0·84	0·84
29 Oct. 1949	300	0·5	1·20	1·20
29 Oct. 1949	300	0·9	0·84	1·32
28 Dec. 1949	300	1	0·72	1·20
28 Oct. 1949	400	0·5	1·84	2·88
28 Oct. 1949	400	1	1·08	1·56
1 Nov. 1949	400	1	1·92	2·28
3 Nov. 1949	400	1	0·60	2·04

It is well known that a shift of the equilibrium between decomposition and resynthesis of adenosine triphosphoric acid in the normally functioning muscle is almost unobservable, even with considerable work [14]. So much the more interesting therefore, in this sense, are the results obtained on resting muscle on the effect of various loads. The experimental results show that a muscle load, just in that range where it evokes an increase in functional properties (i.e. for the frog gastrocnemius muscle up to 200 g) causes an increased resynthesis of adenosine triphosphoric acid.

The muscle load corresponding to the accommodation limit of the tissue (about 200 g) causes so considerable a decomposition of ATP that it just balances its resynthesis and as a result of this compensation no shift in its amount is detected. When the load is increased by another 100 g, a shift in the direction of a predominance of ATP decomposition over its resynthesis is observed, although in one experiment a balance was still observed; this is clearly explained by the individual properties of the muscle. A 400 g load, which is known to exceed the accommodation limit of the muscle, in all cases evokes such a considerable decomposition of adenosine triphosphoric acid that it cannot now be compensated by an increase in its resynthesis. This fact shows that the adaptability of living muscle to external conditions has definite limits beyond which disturbance of "resting" metabolism occurs, accompanied by a decrease in the functional properties of the muscle.

These changes in the content of adenosine triphosphoric acid in a muscle under the influence of various loads agree with the data of Szent-Györgyi *et al.* [15]; they showed a direct relation between myosin solubility and ATP content in muscle, and also the regular beginning of the development of muscle contraction coinciding with the beginning of the decrease in adenosine triphosphoric acid; they showed also the inverse proportionality of the degree of development of this contraction to the amount of ATP remaining in the muscle. In the light of these facts, the increased formation of ammonia in muscle with a load of 400 g and its negligible increase with a load of 200 g, as detected by E. Ya. Geiman, become understandable since the source of ammonia formation in muscle is adenylic acid.

The changes in the other links of muscle metabolism, which take place under the influence of a load, also become meaningful. The increased decomposition of creatine phosphate and the increase in oxidation processes are necessary consequences of the increased decomposition of adenosine triphosphoric acid (the energy of which is used to maintain the excess tension developed by the muscle against the suspended load), for they are the primary sources of its rapid resynthesis. With regard to glycolysis we have the data of Risser and Miura [16] which show that lactic acid production in muscle reaches a maximum just at the mean load; with a much greater load, as with complete removal of the load, it becomes very low.

All these facts show that loading or stretching a muscle up to a strictly determined limit increases its efficiency, because both these factors increase the "resting" metabolism; thus, the free energy of the muscle's contractile structures is increased and this makes the production of much more work possible.

SUMMARY

(1) Depending on its magnitude, a load has a dual effect on the conversion of adenosine triphosphoric acid in muscle.

(2) Under the influence of loads up to 200 g, which heighten the muscle's functional properties, increased resynthesis of ATP in the frog gastrocnemius muscle predominates over its decomposition; this is expressed as an increase in its content in a muscle under these loads.

(3) For loads coinciding with the accommodation limit of the given muscle (about 200 g) the increased ATP decomposition is still fully compensated by its increased

resynthesis; as a result of this a difference in amount of ATP between experimental and control muscle is not observed.

(4) For loads greater than this limit (300 g and more), the considerable ATP decomposition predominates and is now not compensated by resynthesis; as a result its amount in the muscle greatly decreases.

(5) Loading or stretching a muscle up to a certain limit increases its efficiency, for the intensity of the "resting" metabolism increases; thus, the free energy of the muscle's contractile structure increases.

Translated by J. DAINTY

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THE ROLE OF REACTIVE GROUPS IN PROTEIN COMPLEXES IN TASTE RECEPTOR STIMULATION*

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(*Received* 14 *March* 1957)

IDEAS on the roles of certain systems in taste organ reactions have latterly been proposed and the taste receptors have been recognized to be typical chemoreceptors. Baradi and Bourne [1, 2] have shown that various flavoured materials influence the activities of enzymes localized in the taste organs, and that phosphatases and esterases play a particular part. Landgren *et al.* [3] have demonstrated that the acetylcholine-cholinesterase system participates in taste receptor excitation.

In 1920 Pavlov concluded, in relation to the sense of taste, that highly sensitive proteins corresponding to the four types of taste were present in the taste-buds. He

* Biofizika **2:** 6, 665–669, 1957 [Reprint Order No. BIO 88].

considered that the protein split up under the taste stimulus, ionized products being formed which stimulated the nerve-endings [4].

Koshtoyants concluded that reactive groups in proteins, including sulphydryl groups, were important in taste-buds (typical chemoreceptors): this conclusion derived from several studies indicating that protein sulphydryl groups were important in effecting excitation transfer in various elements of the nervous system, in particular in producing afferent excitation in the intestines.

The present work, proposed by Koshtoyants, was intended to check whether reactive protein groups were involved in taste receptor reactions to chemical stimulants.

METHODS

Frogs' tongues were used. The central nervous system was destroyed in each animal. The tongue was removed and fixed to a cork sheet. The tongue was perfused with Ringer's solution via the carotid artery. All stimulants were applied to the dorsal surface with a pipette. Sodium chloride, glucose, picric acid and distilled and tap water were used: Zotterman and Lilestrand [7, 8] have shown these to be active stimulants for the taste receptors. The intervals between tests were strictly constant in any one experiment and were 1–1·5 min. After each taste test (20–30 sec after applying the material) the tongue was washed with Ringer. To the taste receptors this is almost indifferent; it causes only a very slight transient stimulation not repeated on rewashing. Hence, Ringer can be used as a control solution. Oscillographic recording of the action currents from the tongue branch of the glossopharyngeal nerve was used to record the taste sensation. To block the SH groups, $CdCl_2$ was used, a sulphydryl preparation ("unithiol") being used as SH donor. These substances were applied either in moistened tampons placed on the dorsal surface or by injecting them into the perfusion fluid. Concentration of $CdCl_2$: 10^{-2}–5×10^{-4}; time allowed to act: 2–10 min. Unithiol concentration: 1–4 per cent; time allowed to act: 5–30 min.

RESULTS AND DISCUSSION

The results from the perfusion and external application tests were similar, but the effects developed more rapidly on perfusion, indicating that the materials penetrate more quickly to the receptor sites.

The resting-state (background) activity of the nerve was first established (Fig. 1(A); Fig. 2(A)), followed by the activity caused by tap and distilled water as taste stimulants (Fig. 1(B); Fig. 2(B)). The tongue was then washed with SH blocking material ($CdCl_2$) and again tested for taste sensitivity. In all cases the $CdCl_2$ was found to eliminate the sensitivity to the substances, tap and distilled water included. The depression appeared as reduced or completely eliminated nerve activity in response to taste stimulation. The effect developed in 1–3 min after injecting the $CdCl_2$ in the perfusion experiments (Figs. 1 and 2(C)).

Subsequent unithiol treatment restored the sensitivity to normal in nearly all cases. The restoration was sometimes partial, because the blocking agent had destroyed more than just the SH groups. The unithiol restored the sensitivity within 2–7 min of introducing it into the perfusate (Figs. 1 and 2(D) and (E)).

Thus, these taste stimulants, plus tap and distilled water, showed a general regularity; reduced sensitivity in the tongue when the protein SH groups were

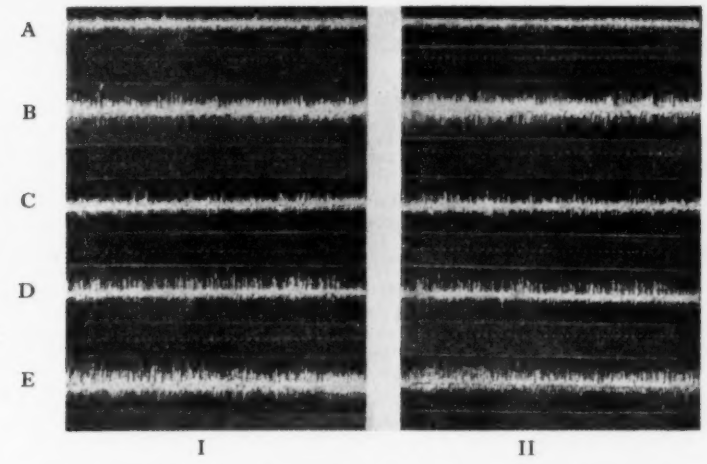

FIG. 1. (A) background activity of tongue nerve; (B) activity on applying distilled water (I) and tap water (II); (C) the same after introducing 0·4 ml of 10^{-3} $CdCl_2$ into the perfusate at 1·5 min; (D) the same after introducing 1 ml of 2% unithiol into the perfusate, at 2 min; the same after introducing 0·5 ml of 2% unithiol (repeat application) after 4 min. Time marks at 0·05 sec intervals.

blocked, and sensitivity restored when the condition of the protein structures returned to normal. These data agree completely with those we previously obtained on dogs. In the latter experiments it was shown that the sensitivity to an acid stimulus (reflex salivation to HCl) was dependent on the SH group state in the taste receptors.

Our data in general confirm Lazarev's hypothesis that highly active proteins take part in taste perception, and Koshtoyants's that reactive protein groups (including SH) are involved in the sensitivities of chemical receptors.

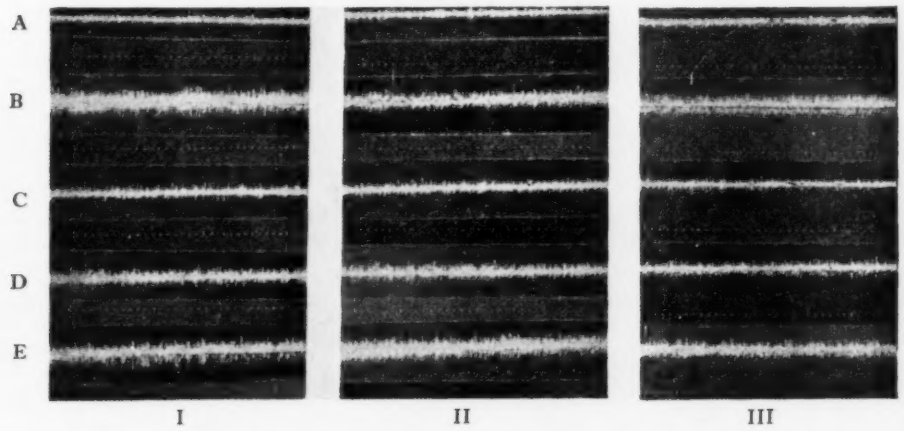

FIG. 2. (A) background activity of tongue nerve; (B) activity on applying 3% glucose (I), 3% NaCl (II), 0·001 M picric acid (III); (C) the same 1·5 min after introducing 0·5 ml of 5×10^{-4} $CdCl_2$ into the perfusate; (D) the same 2 min after introducing 1 ml of 2% unithiol into the perfusate; (E) the same 5 min after the previous unithiol injection. Time marks at 0·05 sec intervals.

CONCLUSIONS

(1) Oscillograph recordings of the activity in r. lingualis n. glossopharyngei showed that an SH-group blocking reagent ($CdCl_2$) depresses the sensitivity of the frog's tongue to taste stimulants (NaCl, glucose, picric acid) and also to tap and distilled water.

(2) Subsequent treatment with an SH-donor (unithiol) gradually restored the sensitivity to the stimulants (including tap and distilled water) which appeared as a rise in nerve activity (frequently to the original value).

(3) Our data in general confirm Lazarev's hypothesis that highly active proteins take part in taste perception and Koshtoyants's that reactive protein groups (including SH) are involved in the sensitivities of chemical receptors.

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THE DEPENDENCE OF *PARAMECIUM* GALVANOTAXIS ON SULPHYDRYL GROUPS*

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(*Received* 14 *March* 1957)

IRRITABILITY, which is the ability of an organism to react to a change in the surrounding conditions, is based, according to modern data, on the special sensitivity of proteins and this is connected with the presence of so-called reactive or functional groups in their molecules. This means that the characteristic phenomena of perception of stimuli, their transformation into excitation energy, and various processes causing response reactions by living matter, can occur on a metabolic basis before the evolution of a nervous system [1].

Wide possibilities for studying the dependence of irritability on the state of protein reactive groups, and in particular on sulphydryl groups which are, on present data, the most labile and reactive, are presented by the simplest organisms, for example, such a comparatively well-studied object as *Paramecium caudatum* which possesses a special form of irritability, galvanotaxis. A change from cathodic galvanotaxis to

* Biofizika **2:** 6, 670–674, 1957 [Reprint Order No. BIO 89].

anodic, due to the blocking of SH-groups of the protein substrate by mercuric chloride, a well-known thiol poison, has been demonstrated on this material in our laboratory. However, when blocking SH-groups by thiol poisons, one could not always avoid exerting a depressor effect on other enzyme systems too; it seemed important to use tissue metabolites which take part directly in blocking SH-groups under the normal conditions of the organism's activity [1]. Therefore, Kh. S. Koshtoyants suggested that we study, on paramecium, the changes in the thresholds and optimal conditions of cathodic galvanotaxis, as they depend on the state of the protein sulphydryl groups, using glutathione, a tissue metabolite which oxidizes sulphydryl groups, and cysteine, a donor of sulphydryl groups.

METHODS

The study of the influence of the reactive groups of proteins on the irritability of paramecia was carried out by the method used earlier (1952) by Popova [2].

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The culture of *Paramecium caudatum* was maintained in Lozina–Lozinskii artificial salt medium with peptone, and also in a hay infusion made from 100 g of hay in 1 l. of water. Yeast and hay bacilli served as food for the paramecia. The yeast was cultured on an agar gel set at a slope. Feeding took place at regular intervals and with definite quantities, for it was necessary to keep the pH of the medium as constant as possible.

Direct current from alkaline accumulators, of voltage 10 V and current from 0·1 mA to 10 A, was used to produce galvanotaxis. Two rheostats were included in order to control the voltage and current in the circuit. Change of voltage was carried out by means of a changeover switch. An ammeter and voltmeter were included in the circuit for measuring voltage and current.

Stimulation was carried out by non-polarizing electrodes, consisting of bent glass tubes whose fine tips were filled with a 1 % solution of agar-agar. The tubes were filled with a saturated solution of zinc sulphate and zinc electrodes dipped into them; these were connected to the electrical circuit. The paramecia, in a drop of the medium, were placed in a hollow in a microscope slide. The volume of the drop and the distance between electrodes were always the same.

Usually no significant change was noticed in the strength and density of the current when glutathione and cysteine were added; this is explained by the smallness of the applied concentrations and the fact that the substances used are weak electrolytes.

The concentrations of the solutions applied in the experiments were: for cysteine, 10^{-2}, 10^{-3} and 10^{-4} per cent; for glutathione, 10^{-4} and 10^{-5} per cent. All solutions were prepared in Lozina–Lozinskii salt medium.

EXPERIMENTAL RESULTS

We first performed experiments on the phenomenon of galvanotaxis itself. The data from one such experiment are presented in Table 1.

From this experiment it can be seen that the threshold current strength at which cathodic galvanotaxis of the paramecia is observed, is 2 mA, with a voltage to the rheostats of 10 V, and that the optimum current strength for obtaining cathodic galvanotaxis is 150 mA. At $I = 200$ mA a change from cathodic to anodic galvanotaxis takes place.

TABLE 1. MATERIAL: *PARAMECIUM CAUDATUM*

Voltage (V)	Current (mA)	Sign of galvanotaxis	Note
	0·8	Nothing	
	2	+	Culture in Lozina–Lozinskii medium
	15	+	— indicates anodic galvanotaxis
	60	++	+ indicates cathodic galvanotaxis
	80	++	
	100	++	
10	150	+++	
	180	++	
	200	Stationary	
	240	—	
	400	——	
	150	++	
	2	+	

After these preliminary experiments the effect of glutathione and cysteine on the behaviour, with no electric field, of the infusoria was studied by adding these substances to a drop of the medium containing the infusoria. As a result of these experiments it became clear that the minimum concentration, at which a change in the motion of *Paramecium caudatum* was observed, was 10^{-6}, for glutathione; the maximum concentration, at which a reversible effect was still observed, was 2×10^{-3}, for gentathione; the corresponding concentration for cysteine was 10^{-3}.

We were able to observe, during the action of glutathione, changes in the body shape and motion of the paramecia which fully agreed with the observations of M. F. Popova on the effect of thiol poisons (mercuric chloride). These phenomena are presented in Table 2.

As can be seen from Table 2, the movement of the paramecia slowed down 3 min after applying glutathione at a concentration of 10^{-5}, and in 7 min the infusoria stopped and their bodies became round. A drop of 10^{-4} cysteine solution led to the restoration of the normal body form and to the restoration of, at first, a slow, and then the usual, motion of the paramecia.

After the above preliminary series of experiments, the fundamental experiments

TABLE 2. MATERIAL: *PARAMECIUM CAUDATUM*

Time hr	Time min	Substance	State of material	Note
12	10	—	Quietly swimming around	Culture is in Lozina–Lozinskii medium
12	16	—	Quietly swimming around	
12	20	Glutathione, 10^{-5}	Quietly swimming around	
12	23		Motion slowed down	
12	25		Motion and rotation slowed down	
12	27	Cysteine, 10^{-4}	Body rounded	
12	31		Slow motion	
12	33		Slow motion	
12	35		Quietly swimming in all directions	

on the dependence of the threshold and optimum conditions of galvanotaxis on the state of the protein SH-groups were carried out. During these experiments we succeeded in tracing an increase in the irritability threshold when low concentrations of glutathione were applied and in obtaining the initial magnitude of the threshold after introduction of SH-groups. The results of one of the experiments are presented in Table 3.

TABLE 3. MATERIAL: *PARAMECIUM CAUDATUM*, 3 DECEMBER 1952

Time hr min	Voltage (V)	Current (mA)	Sign of galvanotaxis	Substance	Note
10 15		0·3	None		+ indicates cathodic galvanotaxis
10 16		0·5	+		
10 17		0·7	+		
10 18		0·5	+		— indicates anodic galvanotaxis
10 19		0·5	+	Glutathione, 10^{-5}	
10 20		0·5	—		
10 21		0·7	—		
10 22		6	+		
10 23	10	1	—		
10 24		6	—		
10 25		20	+	Cysteine, 10^{-4}	
10 26		20	—		
10 27		20	—		
10 28		20	+		
10 29		20	+		
10 30		10	+		
10 31		10	++		
10 32		5	++		
10 33		1	+		
10 34		0·5	+		

TABLE 4. MATERIAL: *PARAMECIUM CAUDATUM*

Voltage (V)	Current (mA)	Time hr min	Substance	Sign of galvanotaxis	Note
10	50	11 00		+	+ indicates cathodic galvanotaxis
	70	11 01		+	
	100	11 02		++	
	150	11 03		+++	Culture in Lozina–Lozinskii medium
	170	11 04		++	
	200	11 05		++	
	150	11 06		+++	
	150	11 07	Glutathione, 10^{-6}	+++	
	150	11 08		+	
	185	11 09		++	
	150	11 10	Cysteine, 10^{-5}		
	150	11 11		+	
	150	11 12		+	
	170	11 13		+	
	150	11 14		+	
	150	11 15		+++	
	150	11 16		+++	

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Thus, from the cited material of the experiments it can clearly be seen that the introduction of glutathione led to an increase in the threshold of cathodic galvanotaxis of paramecium from 0·5 to 20 mA. Cysteine (10^{-5}), introduced on the background of the action of glutathione, led to a lowering of the threshold to the former level.

During the experiments we were also able to establish that the optimum conditions for galvanotaxis were shifted by the oxidation of the SH-groups by glutathione and this shift was restored by cysteine.

In Table 4 data are given from which it can be seen that, under the influence of glutathione, a shift in the optimum conditions from 150 to 185 mA is observed. Introduction of cysteine leads to the restoration of the previous conditions.

CONCLUSIONS

We can draw the following conclusions from the experiments described above.

(1) The galvanotaxis of paramecia is distorted in the presence of glutathione and re-established when free SH-groups are introduced.

(2) When glutathione acts on paramecia, an increase in the threshold of cathodic galvanotaxis is observed; introduction of SH-groups in the form of cysteine leads to a lowering of the threshold of cathodic galvanotaxis to the original value.

(3) In the presence of glutathione a shift of the optimum conditions for cathodic galvanotaxis of paramecia takes place in the direction of higher currents; introduction of cysteine restores the original conditions.

(4) The data obtained enable us to propose that the irritability and metabolism are one in paramecia and that it is possible to control the level of irritability in living matter by acting on metabolic links.

Translated by J. DAINTY

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SIGNIFICANCE OF THE LUNGS AS HEAT-PRODUCING ORGANS*

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(*Received* 14 *March* 1957)

INTRODUCTION

THE development of warm blood in animals is the most recent stage of evolution in the animal kingdom [1–3]. Animals which respired solely via the lungs became warm-blooded. There is an observable sequence in the development of lung respiration and warm-bloodedness.

* Biofizika **2**: 6, 675–685, 1957 [Reprint Order No. BIO 90].

But this form of respiration, while facilitating more rapid vital processes, entailed also the hazard that the animal would be cooled by respiration under bleak climatic conditions. The temperature difference between the blood and inspired air can be as high as 80°C or more in representative arctic fauna [4]. The continuous contact between the cold inspired air and the enormous alveolar surface would inevitably cool the blood passing through the lungs if these did not have also the function of producing heat.

The above developmental sequence and the thermal homeostasis in cold climates indicate that the phylogenetic connexion above is not merely a biological regularity but derives from a more general physical law. The nature of the heat-producing processes giving thermostasis in cold surroundings naturally comes in question. Analysis of the conditions for thermal equilibrium in a thermostatic system indicates that a warm-blooded animal must have a special heat-producing organ which operates when the air is cold and which does so while doing no work. Physiological data indicate the lungs as this organ.

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HEAT PROCESSES IN A HOMOIOTHERMAL SYSTEM

Let us take a warm-blooded animal as our system, and assume it does work continuously at a fixed temperature which exceeds the fluctuating temperature of the surrounding medium to a greater or lesser extent. This system can keep at the same temperature, in excess of its surroundings, and continuously do work of various types only if energy enters it in the form of chemical energy in food. The chemical energy and the work into which it is transformed can be expressed as the product of rate and capacity factors [5]. The transformation between chemical energy of food and working processes of various types can be expressed as follows:

$$\sum q_i m_i = \sum X_i x_i \tag{1}$$

where q_i is the chemical potential, m_i the mass of the food, X_i the rate factor and x_i the capacity factor of some working process.

Suppose the system, being in thermal contact with the surroundings, works at temperature T_N', which is above the surrounding temperature T_s. If simple thermal conduction between system and surroundings occurs, the energy balance for the system is described by the following expression:

$$\sum q_i m_i = \sum X_i x_i + k(T_N' - T_s) + \bar{C}_v \, dT_N'/dt \tag{2}$$

where $k(T_N' - T_s)$ is the heat flow from the system to the surroundings, k being a coefficient of proportionality, $\bar{C}_v$ the mean thermal capacity of the system and dT_N'/dt the time rate of temperature change in the system. The chemical energy is transformed to work with some efficiency e_i:

$$X_i x_i / q_i m_i = e_i. \tag{3}$$

Let us assume that any change in thermal conditions related to changes in $k(T_N' - T_s)$ and $\bar{C}_v \, dT_N'/dt$ is only due to heat liberated when food energy is transformed in the working processes. Then (3) and (2) give

$$\sum X_i x_i \left[\frac{1 - e_i}{e_i}\right] = k(T_N' - T_s) + \bar{C}_v \frac{dT_N'}{dt}. \tag{4}$$

Since $\Sigma X_i x_i$ and T_s are not generally constant T_N' will also not be constant and the system temperature will depend on the working process rates and T_s. The temperature variations are allowed for by the second term, in which the time-derivative can be positive or negative. If we assume the system homoiothermal, i.e. $T_N'=T_N=$ const., or $dT_N'=dT=0$ we must then have

$$\sum X_i x_i \left[\frac{1-e_i}{e_i}\right] \pm Q = k(T_N - T_s) \tag{5}$$

where $\pm Q$ is the heat produced or lost by the system at constant temperature T_N, which depends on the working process rates and T_s. Equation (5) thus gives a general condition for thermal equilibrium in a homoiothermal system. It implies that:

(1) The system must have a special heat-removing organ which disposes of heat $-Q$ at constant temperature T_N when the working process rates and T_s are such that the system would heat up.

(2) It must also have a special heat-producing organ which produces heat $+Q$ to maintain T_N when the working process rates are such (T_s being low) that the system would cool down. The quantity $-Q$ is a latent heat of evaporation Q_e which ensures Td being constant although extra heat is being produced:

$$-Q = -Q_e. \tag{6}$$

Such systems do in fact have heat-removal organs, i.e. the lungs and sweat glands,* which remove heat by evaporating water (phase change) at constant temperature T_N and thus prevent any temperature rise. The heat produced by chemical transformations on some foods is $+Q$, and this maintains T_N when the working organs produce less heat,

$$+Q = \sum q'_i m'_i \tag{7}$$

q and m being as above.

Thus, homoiothermal organisms must have a special heat-producing organ which consumes an amount $\Sigma m'_i$ of food material when the working organs produce insufficient heat or T_s is low, and the body would be cooled. It will be shown that the lungs have this function in warm-blooded animals. The lungs thus have a double function. They remove heat by evaporating water when too much heat is produced in the body, and produce some heat when too little is produced by the other working organs. We then have three formulae for the heat balance of a warm-blooded animal in terms of T_s and the working process rates:

(1) If T_s is high (T_h), i.e. above the comfortable temperature range T_c, the warm-blooded animal must lose heat as latent heat of evaporation ($-Q_e$) via the lungs (and sweat glands), and we have

$$\sum X_i x_i \left[\frac{1-e_i}{e_i}\right] - Q_e = k(T_N - T_h). \tag{8a}$$

If the organism is working hard and T_s is high this heat loss must be appropriately high to prevent overheating.

(2) If $T_s=T_c$ no extra heat loss via evaporation is required, or extra heat production, so we have

$$\sum X_i x_i \left[\frac{1-e_i}{e_i}\right] = k(T_N - T_c). \tag{8b}$$

* Not all warm-blooded animals have sweat glands.

When $T_s = T_c$ the heat required is on the whole produced by the physical work done by the organism.

(3) If T_s is low (T_l), i.e. below T_c, extra heat must be produced by the special heat-producing organ, so we have:

$$\sum X_i x_i \left[\frac{1-e_i}{e_i}\right] + q'_i m'_i = k(T_N - T_l). \tag{8c}$$

If little work is being done and T_s is low this heat must be suitably high. Hence the heat flux at a (constant) low temperature must be constant, i.e.

$$\sum X_i x_i \left[\frac{1-e_i}{e_i}\right] + q_i' m_i' = \text{const. when } T_l = \text{const.} \tag{8d}$$

The total heat produced by lungs and working organs must be constant at T_l; the more produced in the working organs the less in the lungs, and conversely. If much work is being done no extra heat is required from the lungs, although T_s is low, and we get (8b) instead of (8c), with T_c replacing T_l. If still more work is done the heat produced may be in excess of that required for thermostasis. Heat is then lost by evaporation although T_s is low, and (8c) goes over to (8a), T_k replacing T_l. The above formulae thus reflect all possible conditions.

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This analysis indicates that special heat-producing processes unrelated to working ones occur in warm-blooded animals. The special heat-producing organ only functions when $T_s < T_c$ and the heat produced by the working organs is inadequate. Whether such an organ actually exists can only be established by experiment. This conclusion on the heat-producing organ does not complete the analysis, however. If we assume the heat balance to be established in accordance with equations (8a–d) additional conclusions, which can readily be checked by experiment, can be derived.

(1) The special organ cannot simultaneously be a working one, which must be reflected in its morphophysiological structure.

(2) The food source used to produce the heat must be of high calorific value.

(3) The food source used to produce the heat, which is not used by the working processes, should have no specific dynamic action.

(4) The specific dynamic action of other food sources can, according to (8b), only be observed at around T_c.

(5) The specific dynamic action does not occur when T_s is low, because (8d) shows the heat flux to be constant.

(6) The basal metabolic rate at rest without food must be defined by (8b) and (8c) and be higher when T_s is low than when $T_s = T_c$.

We now consider the experimental data which test these deductions.

ENERGY SIGNIFICANCE OF LUNG FAT METABOLISM

Binet and Roger demonstrated lung fat metabolism [6–8]. Now much data has accumulated to show that the lungs store and split fats and oxidize fatty acids [9–13]. These functions are coupled to liver activities; the lungs supplement and balance liver fat metabolism [14]. Active fat metabolism is an additional function of the lungs which is indicated by much evidence. This function has so far received no physiological explanation. Its significance is apparent because the lungs have no appreciable

part in protein and carbohydrate metabolism. This has been demonstrated by experiment. The total and residual nitrogen contents in arterial blood and blood from the right-hand side of the heart are identical [15]. The sugar, glycogen and lactic acid contents of the blood of a non-starving animal do not change to any appreciable extent on passing through the lung circulation, so lungs have no part in carbohydrate exchange between organs [16, 17].

The data show, therefore, that the lungs are active in fat metabolism in a non-starving warm-blooded animal, but not as regards proteins and carbohydrates. This can be interpreted as an indication that the lungs require fats for heat production and not for performing work. We may wonder why the lungs produce the extra heat required at low temperatures. The reason is that in warm-blooded animals the moist lung surface is enormous on account of the ramified capillary system [18]. To prevent the blood passing through the lungs being cooled by contact with the cold air the lungs must produce heat. The fat consumption protects the lungs from cooling due to the continuous gas (H_2O and CO_2) loss, which goes to heat the air. That the lungs use fats and not glucose confirms our second deduction, namely that the source must be of high calorific value.

RELATION OF LUNG FAT METABOLISM TO TEMPERATURE OF SURROUNDINGS

The lungs produce heat by oxidizing fats when T_s is low. We have found no experimental data on the relation of lung fat metabolism to T_s in the literature. Seasonal variations in blood lipids have been observed in healthy humans. These few observations indicate a tendency to reduced lipid content in the winter months [19, 20]. We may suppose this related to lung fat oxidation. Fats mobilized from the fat depots are transferred by the blood to the lungs and there oxidized. Experimental studies on warm-blooded animals to establish the lung lipid content as a function of T_s are of interest.

EXPERIMENTAL

The experiments were done on warm-blooded animals in the winter of 1955–56. Rats, guinea-pigs and cats were used. The animals were divided into two groups. One group was kept at a low temperature (4–6°C) for 3–6 days, the other at 25–28°C. There were 10 series, each series containing one cold and one warm group. The animals were fed normally (same for all groups). After this period the animals were decapitated and the lungs dissected out. In all, 65 animals were used: 28 cold and 29 warm rats, 2 cold and 2 warm cats, and the same number of guinea-pigs.

The lungs were kept in a drying oven at 95°C for 10 hr. The dried lungs were powdered and dried to constant weight. After cooling the powders were transferred to previously defatted paper thimbles which had been dried to constant weight. The thimbles (loaded and unloaded) were weighed to $\pm 0{\cdot}2$ mg on analytical balances. They were then ether-extracted in a Soxhlet apparatus for 8 hr. The weight loss after extraction gave the lipid content in mg. The percentage loss in weight was calculated.

The root mean square deviations were calculated from

$$\sigma = \pm[\sum d^2/(n-1)],^{\frac{1}{2}}$$

Σd^2 being the sum of the squares of the deviations from the mean and n the number

of determinations (see Table 1). The data in Table 1 show that the cold animals tended to have more lung lipids than the warm. It may be assumed that the air temperature has a reflex action on the lung fat metabolism.

We now consider the deductions made as to the specific dynamic actions of foodstuffs and the temperature variation in the basal metabolic rate.

LUNG HEAT PRODUCTION AND SPECIFIC DYNAMIC ACTIONS OF FOODSTUFFS

Experiment shows that the specific dynamic action of foodstuffs (SDA, for short) depends on T_s and the nature of the foodstuff. Fats either have no SDA at all [3, 21, 22] or only a very slight one compared with proteins and carbohydrates. This has so far not been explained but is in complete agreement with our third deduction (the foodstuff which serves as the lung heat source is not used in working processes and should have no SDA).

Proteins and carbohydrates, unlike fats, have high SDA at T_c. This agrees entirely with our fourth deduction, i.e. that the heat flux increases after taking food because of increased working process activity, and that this can be observed only at about T_c.

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TABLE 1. EFFECT OF SURROUNDING TEMPERATURE ON LUNG LIPID CONTENT

Series No.	Animal No.	Lung weight in mg and % weight loss						
		Cold				Warm		
		A: pre-extraction weight	B: post-extraction weight	% loss $\frac{A-B \times 100}{A}$	Animal No.	A: pre-extraction weight	B: post-extraction weight	% loss $\frac{A-B \times 100}{A}$
1	2	3	4	5	6	7	8	9
I (rats)	1	278·0	246·0	11·5	6	210·8	192·8	8·5
	2	339·7	298·3	12·2	7	313·0	287·0	8·3
	3	502·4	442·8	11·8	8	239·5	212·6	11·2
	4	312·9	273·8	12·5	9	380·0	338·0	11·0
	5	393·5	341·5	13·2	10	312·6	288·0	7·9
	Mean	. . . .		12·2				9·4
		σ . .		± 0·66				± 1·59
II (rats)	1	253·8	209·6	17·4	3	505·6	443·3	12·3
	2	252·2	214·6	14·9	4	392·0	346·9	11·5
					5	270·9	229·6	15·2
	Mean	. . . .		16·2				13·0
III (rats)	1	246·7	217·8	11·7	5	240·0	215·6	10·2
	2	338·6	302·3	10·7	6	244·1	222·2	9·0
	3	284·1	204·1	9·9	7	291·9	269·6	7·6
	4	359·2	323·0	10·1	8	301·1	275·0	8·7
					9	274·5	247·5	9·8
	Mean	. . . .		10·6				9·1
		σ . .		± 0·31				± 0·81
IV (cats)	1	1025·8	881·2	14·1	3	1057·2	934·3	11·6
	2	1064·4	942·0	11·5	4	1215·6	1071·1	11·9

TABLE 1—continued

Series No.	Animal No.	Lung weight in mg and % weight loss: Cold A: pre-extraction weight	Cold B: post-extraction weight	Cold % loss $\frac{A-B}{A}\times 100$	Animal No.	Warm A: pre-extraction weight	Warm B: post-extraction weight	Warm % loss $\frac{A-B}{A}\times 100$
1	2	3	4	5	6	7	8	9
	Mean . .	. .	. .	12·8				11·8
V (rats)	1	181·7	158·3	12·8	5	306·0	269·3	12·0
	2	270·0	234·4	13·2	6	288·2	250·3	13·2
	3	249·3	214·5	13·9	7	282·0	246·3	12·7
	4	296·3	258·5	12·7	8	212·9	184·7	13·4
	Mean . .	. .	. .	13·2				12·8
	σ . .	. .	. .	± 0·54				± 0·53
VI (rats)	1	215·3	197·2	8·4	4	148·3	137·1	7·6
	2	241·3	222·7	7·7	5	255·1	240·9	5·6
	3	186·8	175·6	6·0	6	169·3	159·8	5·7
	Mean . .	. .	. .	7·4				6·3
VII (rats)	1	183·0	169·7	7·8	4	122·9	113·8	7·4
	2	168·6	155·3	7·9	5	190·7	178·1	6·5
	3	204·7	185·8	9·2	6	269·1	256·8	4·6
	Mean . .	. .	. .	8·3				6·2
VIII (rats)	1	311·9	286·4	8·1	5	198·9	183·8	7·6
	2	227·2	210·8	7·2	6	215·7	198·3	8·1
	3	168·8	152·1	9·8	7	241·0	220·8	8·3
	4	103·3	91·4	11·7	8	328·1	298·2	9·1
	Mean . .	. .	. .	± 9·2				8·3
	σ . .	. .	. .	1·98				± 0·82
IX (guinea-pigs)	1	641·5	560·6	12·6	3	1318·7	1203·3	8·8
	2	886·2	772·5	12·8	4	781·6	699·1	10·6
	Mean . .	. .	. .	12·7				9·8
X (rats)	1	330·9	293·0	11·4	4	260·9	232·7	10·8
	2	224·3	200·6	10·6	5	272·4	245·0	10·0
	3	265·3	236·9	10·7				
	Mean . .	. .	. .	10·9				10·4

As regards the relation of SDA to T_s, the percentage increase in basal metabolic rate falls sharply with T_s. This agrees with our fifth deduction: SDA does not exist when T_s is low, since the heat flux is then constant.

The basal metabolic rate drops sharply as the air temperature rises. This agrees with our sixth deduction: the basal metabolic rate of a warm-blooded animal must be higher at low temperatures than in the comfort zone.

The data thus confirm all our deductions, and the agreement between theory and experiment indicates, in our opinion, that this theory correctly represents reality.

CONCLUSIONS

(1) Warm-blooded animals have a special heat-producing organ, the lungs, which produce heat when the surrounding temperature is below the comfort range, and when the heat produced by working processes is insufficient to maintain the body temperature.

The heat balance in a homoiothermal system at low temperatures and low working process rates is described by

$$\sum X_i x_i \left[\frac{1-e_i}{e_i}\right] + q'_i m'_i = k(T_N - T_l)$$

where $\Sigma X_i x_i$ is the sum of all forms of physical work done in the basal metabolic condition, the e_i are the efficiencies, the q'_i are the chemical potentials and the m'_i the masses of foodstuffs (fats) used to produce heat in the lungs without doing any work. The heat flux is given by $k(T_N - T_l)$, where k is a coefficient of proportionality, T_N is the system temperature and T_l is the surrounding temperature (below the comfort range).

(2) During evolution, animals which have come to rely on lung respiration alone have become warm-blooded. On adaption to cold climates the lungs have acquired the additional function of producing heat. When the surrounding temperature and working process rates are low the lungs produce heat from fats without doing any physical work. The lungs thus form a heat-producing system which is operative when certain internal and external conditions operate on the system.

(3) The low SDA of fats is due to their being used by the lungs to produce heat.

(4) The lung heat production falls after taking food to an extent which balances the increased heat resulting from metabolic processes in other organs. This explains why the SDA of food vanishes when the surrounding temperature is low.

(5) The lung lipid content tends to rise as the surrounding temperature falls.

Translated by J. E. S. BRADLEY

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PROPAGATION OF NON-IMPULSE EFFECTS ALONG A NERVE FROM A REGION EXPOSED TO X-IRRADIATION*

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(*Received* 10 *April* 1957)

UNTIL recently it was widely supposed that the nervous system was highly resistant to ionizing radiations, an assumption which partly accounted for the failure to appreciate the significance of the nervous system in the development of radiation injury. However, this concept can scarcely be reconciled with the extensive data in medical and radiobiological literature at present.

Tarkhanov [1, 2] and a number of other investigators [3–6] showed that ionizing radiations affect the state of the central nervous system, and their work was followed by that of Nemenov [7, 8] and other Soviet authors [9–11] who were able to demonstrate that changes occur in the conditioned reflex activity of animals following irradiation of the brain. Recently, changes have also been demonstrated in the electrical activity of the cerebral cortex [12–14] and also in the impedance of cerebral cortical matter [15, 16] on exposure of the head to X-rays and ^{60}Co γ-rays. A number of findings indicate that when the diencephalon region is irradiated variations appear in the most important vegetative functions (gastric functioning, cardiovascular system) and in the indices of metabolism and blood composition, etc. [17, 18]. Finally, local or regional changes at the periphery on irradiation of limited areas of the central nervous system [19–21] have been demonstrated and proof furnished that these changes are the result of a spread of effects along the nerve formations themselves and not by means of a system of circulation in the organism.

Irradiation of the various peripheral formations and, in particular, of the receptor mechanisms, produces not only functional changes in the irradiated part itself, but

* Biofizika **2:** 6, 686–697, 1957 [Reprint Order No. BIO 91].

also affects the activity of the central nervous system [22, 23]. It is usually assumed that variations in the functioning of the central nervous system are produced by changes in afferent impulsation from the site of the primary action of irradiation [22, 24].

The majority of workers who have investigated the effects of X-rays and other ionizing radiations on nerve formations assume them to be based on changes in the nature of impulse activity arising either centrally or at the periphery. Bakin and Naumenko [20, 25] are the only authors to advance the hypothesis that effects may be spread from the irradiated region by a non-impulse mechanism, but they give no concrete findings to confirm that such changes occur along the nerve paths and links.

It has not been proved that nervous impulses constitute the sole means of transmission of the effects originating at the site of irradiation in all cases where these may be assumed to pass along nerve structures. If this were so it should be expected that the remote centripetal or centrifugal effects ought to be entirely a matter of an intensification or weakening (to the point of complete disappearance) of the impulsation. However, certain phenomena observable on irradiation, for example, those described by Bakin and Naumenko and others [20, 25] are difficult to explain on this basis.

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It is highly unlikely that changes due to X-irradiation in structures such as the body of a neurone or an axon fail to exert some type of constant influence on the adjacent or more remote parts of the nerve cell structure, if account be taken of their continuity, their "linear" arrangement and the fibrous nature of the axoplasm as confirmed by modern electron microscopic investigations.

Kirzon has advanced the hypothesis that the local action of X-rays on a nerve trunk must cause functional changes over a considerable length analogous to the effect observed on subjecting a nerve to chilling, heat, a direct current, or to certain ions of importance for the function of conduction or for the state of the neural conductor, or to other factors [26–31]. In other words, it has been suggested that the effects of X-rays are spread along neural conductors by a non-impulse mechanism. The theory of non-impulse effects, propounded by Vvedenskii, based in part on the phenomenon which he termed "perielectronic" [32], was later elaborated by Ukhtomskii and by other pupils and followers [33–35]. According to this theory both impulse and non-impulse mechanisms operate in the nervous system and in its relations with the periphery.

Kirzon together with Kol's [36] presented further proof that in the paradoxical stage of development of parabiosis in a nerve-muscle preparation, non-impulse effects spreading along the nerve path also involve neuro-muscular synapses as a result of which the phenomenon of a persimum of force may develop in the synapses before such phenomena become distinct in the parabiotic region itself.

Subsequently Kirzon and his co-workers Kol's, Tsukerman; Prudnikova; Smirnova, etc. [37–39], presented further data suggesting that changes, produced initially in a limited part of a nerve trunk by the action of novocain, cocaine, or the anode or cathode of a sub-threshold direct current, etc., are transmitted along nerve formations by means of non-impulse effects. Since functional changes were observed in the nerve trunks of the opposite side it had to be recognized that non-impulse effects can reach the central synapses as well as the peripheral neuro-muscular ones, passing

through the synapses to the posterior neurones. These findings led Kirzon to the idea that a pathological process (e.g. an inflammatory one) developing in such a way that it involves even a small part of the nerve cell (body of the neurones or their axons) must produce changes in the functional state which spread extensively and may involve both central and peripheral synapses. On the basis of all the established phenomena on spread of non-impulse effects, Kirzon has advanced the hypothesis that local changes in nerve formations resulting from X-irradiation may also be the source of changes which spread far along the nerve structures involving both the synaptic and post-synaptic structures.

In order to investigate the non-impulse effects arising upon local irradiation of nerve formations, it was most convenient to utilize the classic object—a frog nerve-muscle preparation—which has long served as a model in investigations both of simple and complex nervous phenomena.

It has long been established [40–43] that a number of distinct functional changes occur in the part of the nerve trunk actually irradiated: bi-phasic change in excitability and progressive fall in conductivity culminating in a total block to the spreading nervous impulses. In agreement with the findings of the school of Tasaki [44], upon local irradiation of a single nerve fibre, a fall in the electrical response of the spreading wave of excitation has also been detected in the nodes of Ranvier [45].

However, no worker investigating the effects of ionizing radiations on a nerve trunk has followed up the changes which, according to the theory of non-impulse effects, ought to be expected at a greater or lesser distance from the irradiated part. The object of our investigations was, firstly, to establish and study in detail the physiological manifestations of the spread of effects by non-impulse mechanisms from the irradiated region along the nerve trunk and, secondly, to make clear whether or not these non-impulse effects spread to muscular formations including neuro-muscular synapses.

METHODS

Changes in the excitability of the nerve were traced by the usual myographic technique. Observations were conducted simultaneously on two nerve-muscle preparations, consisting of the sciatic nerve and gastrocnemius muscle of the frog (*Rana temporaria*), one preparation serving as the test and the other as control. A small length of nerve in the test preparation—8–9 mm—was exposed to X-rays (Fig. 1). Both test and control were placed in a special humid Plexiglas two-compartment chamber. The compartment containing the control was reliably protected from penetration of X-rays during irradiation of the test by means of a lead, aluminium and Plexiglas partition (Fig. 1). The test nerve was placed on two pairs of stimulating electrodes I and II prepared from silver wire of 0·75 mm diameter. In some experiments an extra pair of electrodes III was interposed between electrodes II and the muscle. The electrode pairs I, II and III were respectively 10, 15 and 25 mm distant from the irradiated region. Electrodes I were on the proximal part of the nerve and served as a means of investigating the excitability of this section of the nerve trunk and conductivity through the irradiated section. A specially constructed generator of rectangular pulses, the duration, frequency and amplitude of which could be changed independently, served as the source of test stimuli. The pulse frequency in all the experiments was 50 c/s and the duration of each pulse 0·7–1·0

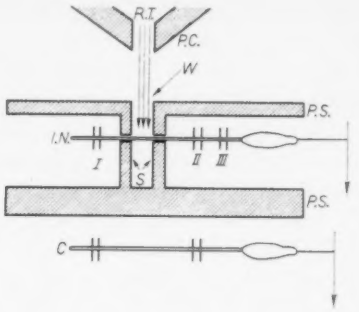

FIG. 1. Diagram of chamber containing preparations. IN—irradiated preparation; C—control; I, II and III—stimulating electrodes; RT—X-ray source; PC—protective metallic casing of X-ray set; PS—lead and aluminium protective screen of chamber; S—lead and aluminium protective shields; W—window in protective screen PS.

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msec. The direction of the stimulating current was always descending, and stimulation was performed at 30 sec intervals. The muscles were arranged vertically and their contractions recorded in isotonic conditions on Engelman myographs. The load on the muscle with equilibrated arms of the small lever was about 12–14 g.

We endeavoured to ensure that irradiation of the test nerve was as localized as possible (8–10 mm in length) and therefore, despite the fact that at the exit of the tube the beam of X-rays was narrow enough (9–10 mm), we lined the compartment

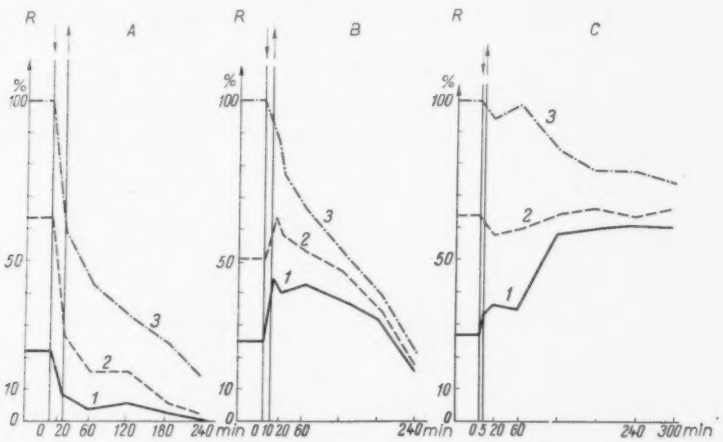

FIG. 2. Changes in level of muscular contractions on stimulation of irradiated nerve in proximal section (see electrodes I in Fig. 1). Abscissa—time from commencement of irradiation in minutes; ordinate—arithmetrical mean of magnitude of muscular contractions in various experiments expressed in percentages. (In each experiment the magnitude of the maximum muscular contractions before irradiation is taken as 100 per cent.) Lines indicate muscular responses to (1) threshold, (2) average and (3) maximum stimuli. *R*, together with the arrows, denotes commencement and end of irradiation. Graph A—20 min irradiation; Graph B—10 min and Graph C—5 min.

containing the test preparation on the side facing the X-ray tube with lead and aluminium, leaving a small window, 10×10 mm (Fig. 1, W). In order to exclude the effects of dispersed X-rays and possible secondary irradiation of the parts of the nerve lying immediately outside the limits of the irradiated segment, two lead and aluminium shields (Fig. 1, S) were set up in the chamber parallel to the beam of X-rays and at right angles to the nerve, fine apertures being made in them to take the nerve. The control preparation was securely protected by a thick lead screen (Fig. 1, P.S.). For irradiation we used a universal X-ray tube (URS-70 K 1), as employed in spectrum analysis, run at 30 kV, 14 mA, without filters. The focal distance in all experiments was 2 cm. Dosage was determined by time of irradiation which in the various experimental series was 5, 10 and 20 min. One-minute irradiation corresponded to a dose of 400–450 r.

The experiments were conducted as follows: before irradiation recordings were made of the initial muscular responses to different strength stimuli in both test and control. In accordance with the magnitude of muscular contractions obtained, three strengths of stimuli were selected, corresponding to threshold, average and maximum, for each point along the nerve. The subsequent changes in muscular response to these three different strength stimuli were the criteria of variations in excitability at the different points along the nerve or of conductivity in the irradiated section and in the neuro-muscular synapses. These test stimuli were repeated at definite time intervals (10–20 min) throughout the 4–5 hr experiment.

From the results of pilot experiments we selected three different periods of irradiation (20, 10 and 5 min) for which three series of observations were made. Such dosages enabled us in observations lasting several hours to detect slight variations in excitability which might have escaped notice with use of larger doses.

EXPERIMENTAL RESULTS

Despite the different rate of change in the individual preparations for a given dose of radiation, it was clearly seen that effect was basically dependent on radiation dose.

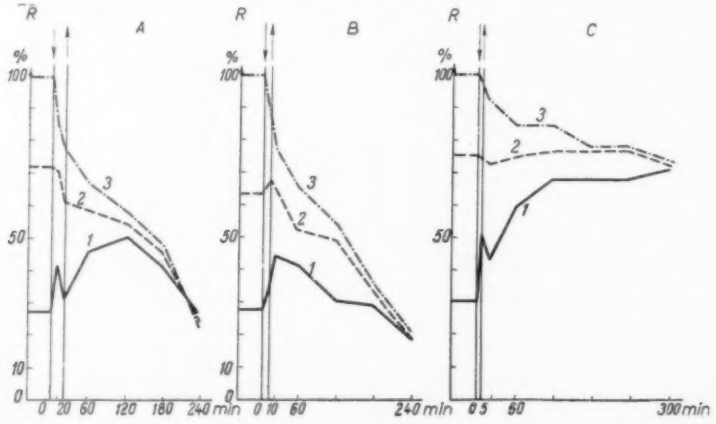

FIG. 3. Changes in level of muscular contractions on stimulation of irradiated nerve in distal section (see electrodes II in Fig. 1). Symbols as in Fig. 2.

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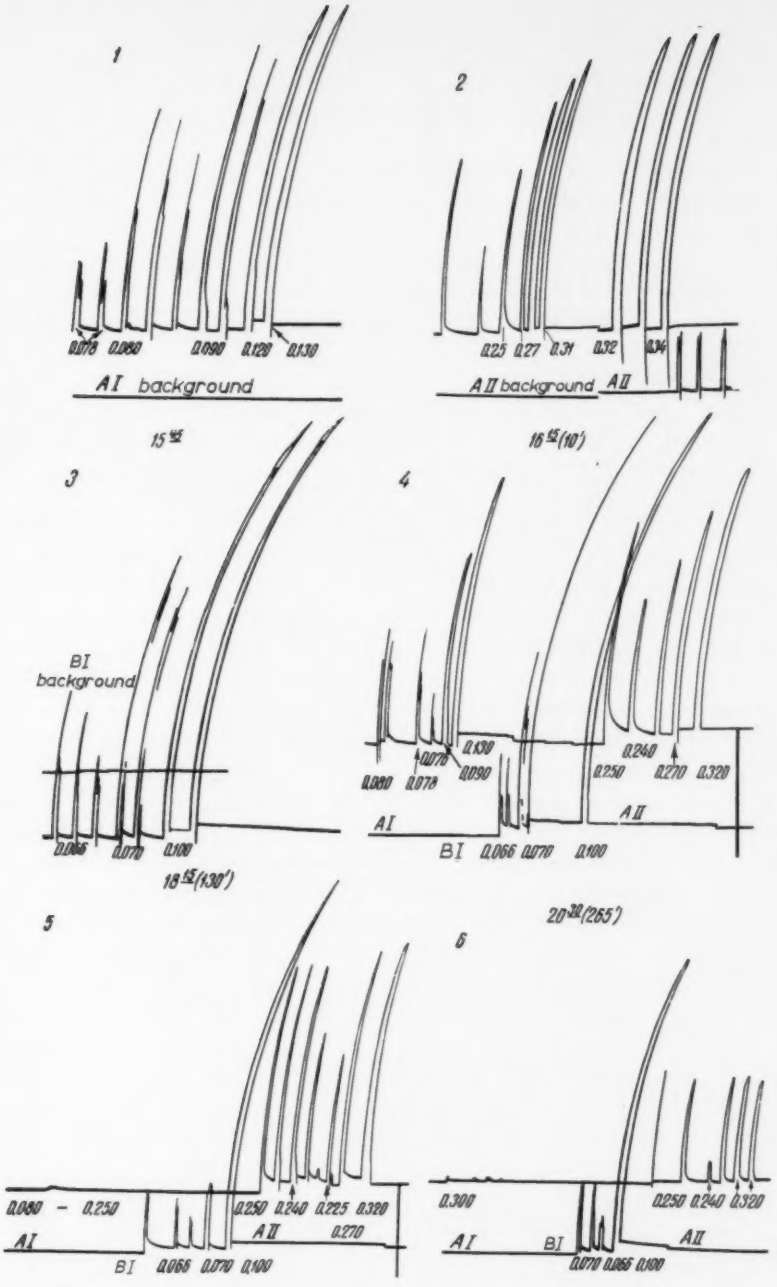

FIG. 4. Kymographic tracings obtained in experiment in which part of nerve received 20 min irradiation. (Experiment No. 52.) Upper row—record of contractions of muscles in irradiated preparations: A1—on stimulation of nerve at electrodes I; A2—at electrodes II (see Fig. 1). Lower row: record of contractions of control muscles; B1—on stimulation of nerve at electrodes I (see Fig. 1). Numerals below records—voltage of stimulation. Figures at top—protocol time; figures in brackets—time from commencement of irradiation (in minutes). 1–3 are before irradiation; 4–6 are at various time intervals after irradiation.

A feature common to all doses was that, after irradiation lasting 5 min, or, of course, for longer periods, changes were observed which continued to develop for 3–4 hr after irradiation.

In view of the fundamental differences in the results with stimulation at electrodes I (on the proximal part of the nerve) and electrodes II (in the distal part), it is necessary to consider these results separately.

Figs. 2, 3 and 5 depict composite graphs of the changes in muscular response of the irradiated and control preparations, plotted from the data of each series of experiments in which irradiation time differed. Specimen tracings are presented in Fig. 4.

Changes in the response of the muscle to stimulation in the proximal nerve

The changes in the magnitude of the muscular contractions upon stimulation by electrodes I lying posterior to the irradiated section (Fig. 2) probably express the result of an interrelationship between diminishing conductivity in the irradiated part and enhanced excitability at the very site of stimulation.

When the dose of radiation was large, corresponding to 20 min irradiation (Fig. 2A), a decline in muscular responses to all three test stimuli was found both on cessation of irradiation and in the subsequent period (see also myograms in Fig. 4). From 3 to 5 hr after irradiation the muscles gave practically no response to threshold and average stimuli and the magnitude of the responses to maximum strength stimuli fell to about 20 per cent of the original level. All this is an expression of a progressive decline in the conductivity of the irradiated part, a decline which was protracted in time with the most powerful stimulus as a result of the utilization of the largest number of nerve trunk fibres.

For a smaller dose of radiation (Fig. 2B) the course of the changes in muscular response varied according to the strength of stimuli. With maximum stimuli a progressive diminution in conductivity occurred while average and threshold stimuli gave an initial increased muscular response which then began to decrease but at a slower rate without ever reaching zero. It is noteworthy that in the case of threshold stimuli the muscular responses were inappreciably reduced as compared with their

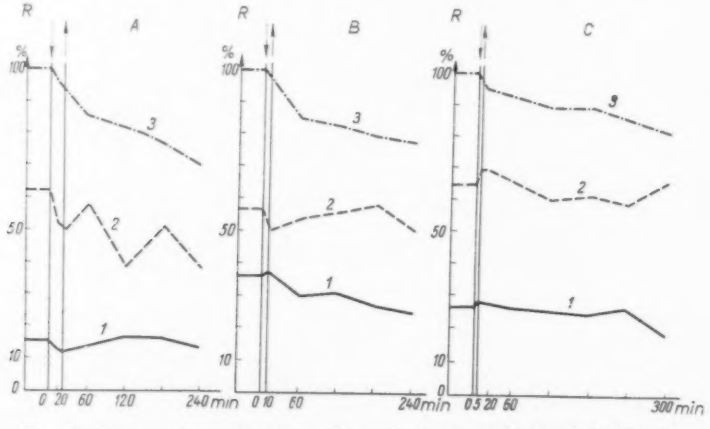

FIG. 5. Changes in contractions of muscles in controls (see C in Fig. 1).

original levels. We cannot fail to note in these differences in muscular contractions, in response to different strength stimuli, relationships analogous to those first described by Vvedenskii [46] in studying the parabiosis phenomenon.

Finally, with small radiation doses (5 min irradiation) (Fig. 2C) there was a continuous rise in the response to threshold stimuli. Correspondingly, the responses to average stimuli remained practically unchanged while with maximum stimuli there was a fall even though it was insignificant (20–25 per cent of maximum response).

Hence we may conclude from the experimental findings that the changes in muscular response to stimulation of the nerve by electrodes I were, on the one hand, due to changed excitability in the non-irradiated proximal nerve and, on the other, to a decline in conductivity in the irradiated zone.

Changes in the response of the muscle to stimulation in the distal nerve

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For all the investigated radiation doses we observed, by applying a weak stimulus to a point 15 mm distal to the site of irradiation (electrodes II in Fig. 1), increased muscular contractions. This was particularly evident when the radiation dose was low (5 min) (Fig. 3C). We deduced that there is increased excitability in the distal section of the nerve which persists for a long time.

However, average and high stimuli produced progressively falling responses which within 3–4 hr of irradiation equalled the responses to threshold stimuli. Superficially this bears a close resemblance to the equalizing stage of parabiosis described by Vvedenskii [46]. When the dose of radiation was small, the muscular responses to different strength stimuli levelled off at about 75 per cent of the original maximum level (Fig. 3C).

Thus, we observed that the irradiated nerve section exerted a two-stage effect on the non-irradiated part. In the first short-lived phase, with use of high radiation doses, a rise was seen in muscular response denoting enhanced excitability in the distal nerve segment. In the second phase a progressive decrease was observed in muscular response which was particularly pronounced with large doses of radiation and stronger test stimuli.

That all the changes described were due to the irradiation was confirmed by means of control investigations carried out simultaneously with the tests. In Fig. 5 (A, B and C) the mean values are given for the changes in response of the control muscles throughout the various series of experiments in which the time of irradiation of the test and also the strengths of stimuli differed. The graphs show only a gradual fall in the magnitude of the contractions which is to be expected in the course of a 4 hr experiment. Additional evidence suggesting that all the changes in the test preparations arose as a result of X-irradiation was the absence in the controls of an increased muscular response to threshold and average stimuli which was so characteristic and consistent for the test preparations when the radiation dose was small.

In a further series of experiments we stimulated the nerve at a point 25 mm distal to the irradiated part (see electrodes III in Fig. 1) and found that the results coincided with those obtained on stimulation at the point 15 mm distal to the irradiated region. Hence we conclude that the excitability of the nerve fibres was increased for some time after irradiation throughout the entire distal part of the nerve from the boundary of the irradiated zone to the muscle. This is an expression of the non-impulse effects

which spread from the irradiated part to the whole distal nerve. It may be confidently affirmed that such a change spreads also along the proximal part of the nerve trunk but that it is dominated by the reduced conductivity of the irradiated part leading to reduced muscular responses.

While it may readily be assumed that the phase of prolonged or even temporary increase in muscular response to weak and average strength stimulation of the distal part of the nerve is a consequence of increased excitability of the nerve fibres, how are we to interpret the uninterrupted decrease in muscular contractions observed with use of large doses of radiation and powerful stimuli ? Since the nervous impulses arising in the distal section of the nerve are not passed through the irradiated section it cannot be supposed that, with use of powerful stimuli, these nervous impulses are blocked to a greater or lesser degree in the irradiated section. This leaves only two possibilities: either the lowered muscular responses are due to "weakening" of excitation (i.e. a reduced number of excited nerve fibres or a transformed rhythm of excitation) at the site of stimulation in the nerve itself; or they must be referred to the peripheral structures (neuro-muscular synapses and the muscle itself). The first of these two possibilities may be discarded for two reasons: firstly, threshold stimuli produced, as we have noted, distinct signs of a prolonged rise in excitability; and secondly, subsequent electro-physiological investigations specially conducted by M. G. Pshennikova (see also [48]) gave direct proof that during the 3–4 hr after irradiation of a small nerve section there was in fact increased excitability in the non-irradiated part of the nerve whatever the strength of the stimuli. Thus, the fall in muscular response, which occurred all the earlier and in a yet more pronounced form the larger the dose of radiation and the more powerful the stimulus, became gradually sharper despite the fact that the increased excitability of the nerve fibres was maintained and even increased.

We believe that this contradiction is only an apparent one. The investigations of Kirzon, together with Kol's and other co-workers, have shown that the non-impulse effects originating from a focus of parabiosis and also from the point of application of strong electric shocks may develop their depressive action on neuro-muscular synapses, thereby causing onset of paradoxical relations for weak and strong nerve stimuli. It is worthy of note that this may occur before the conditions are created for a transformation of the rhythms in the section of parabiosis itself.

In our case it may also be assumed that the non-impulse effects, arising in the irradiated section and spreading along the conducting axon structures with involvement of the neuro-muscular synapses (and perhaps of the muscle fibre itself) probably gave rise earlier to a reduced functional state precisely in the synaptic structures. Such a hypothesis is sufficiently well substantiated if it is borne in mind that a neuro-muscular synapse, as compared with a nerve or muscle, possesses the least functional resistance to different types of action exerted on it.

Both during irradiation and the period after irradiation in which we studied the excitability of the nerve trunk, we observed, as a rule, neither contractions nor fibrillations in the muscle except at the moment of stimulating the nerve. This means that the irradiation process was not the cause of nervous impulses and, consequently, all the processes observed by us were the result of non-impulse effects spread from the irradiated region.

It is important to note that the changes in muscular response, indicating increased excitability in the distal part of the nerve trunk, continued to manifest themselves even when the conductivity of the irradiated section had almost completely disappeared. This must mean that the nerve fibres, which have lost their capacity to transmit waves of excitation, continue to spread effects from the irradiated section, leading to deepening functional shifts in remote parts of the nerve fibre and in the neuro-muscular synapses.

If we admit the spread of effects to neuro-muscular synapses it must be recognized that these effects also reach the nearest synapses in the central nervous system for, in special experiments, we saw that changes are also spread in a proximal direction from the irradiated section. This means, in particular, that the action of X-rays on the receptor mechanisms or on the nerve fibres must cause localized functional shifts in the central nervous system and then in regions of the periphery which are themselves not subject to irradiation.

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Our assertions that non-impulse effects may spread towards the periphery and the central nervous system do not, of course, exclude the possibility that irradiation may affect the capacity of receptor structures to generate impulse excitations, as has been demonstrated by Livanov [22] and also by Geinisman and Zhurmunskaia [47]. Changes induced in the receptor apparatus by irradiation will either lead to an intensified impulsation from the receptor as response to a stimulus or, on the contrary, to a weakening or cessation of this impulsation. But, in addition, the irradiated nerve receptor, as well as the axon, may be a starting point for the spread of non-impulse changes directed towards the central nervous system. Irradiation of the axons themselves (at least with weak or moderate doses) will cause in the first place a spread of non-impulse effects which will be seen as changes in the functional state.

SUMMARY

(1) Following irradiation of a small segment of frog nerve, the muscular responses to stimulation of the nerve at a point proximal to the irradiated part show that for a short period only a rise in excitability takes place. This is followed by a progressive loss of conductivity in the irradiated segment. The rate of development of the block is dependent on radiation dose.

(2) Stimulation in the nerve distal to the irradiated segment indicates that increased excitability develops. This process was investigated at distances of up to 25 mm from the limits of the irradiated segment.

(3) The rate with which muscular responses weakened was dependent on radiation dose and strength of stimuli applied in the distal nerve. Ideas are presented in support of the view that this weakening in muscular responses occurs as a result of a decline in the functional capacities of the neuro-muscular synapses and perhaps of the muscle itself.

(4) Changes in muscular response and the conclusions drawn from them regarding prolonged change in excitability in the distal part of the nerve, allow one to affirm that from the irradiated nerve trunk region non-impulse effects are spread which become more pronounced despite cessation of irradiation and loss of conductivity in the irradiated part.

(5) The hypothesis is advanced that irradiation of peripheral nerve structures (receptors and axons of nerve cells) leads not only to change in the capacity of the receptors to generate nervous impulses in response to adequate stimuli but also to the emergence of non-impulse effects which are spread to the nearest synaptic structures of the central nervous system.

Translated by A. CROZY

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MOTION OF THE EYE ON INTERCHANGING FIXATION POINTS AT REST IN SPACE*

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(*Received* 14 *September* 1957)

THE eye motion was recorded by a method previously described [1]. Seven subjects with normal vision were used in the experiments.

RESULTS

(1) The fixation points (A and B) were white spheres lying relative to the subject in a sagittal plane such that B was further away and somewhat above A. The angular size of the spheres was 2°. The minimum distance from the subject's eyes and A was 15 cm, and the maximum distance of B was 950 cm.

In the first series the motion was recorded on oscillograph paper fixed before the subject. Fig. 1 shows a record from the left eye on shifting the fixation point five times from A to B (here the right eye record is a mirror image of the left one with respect to the sagittal plane). The records similar to that of Fig. 1 all show that the transfer from A to B always occurs in the same way. At first there is a slight divergence

* Biofizika **2:** 6, 698–702, 1957 [Reprint Order No. BIO 92].

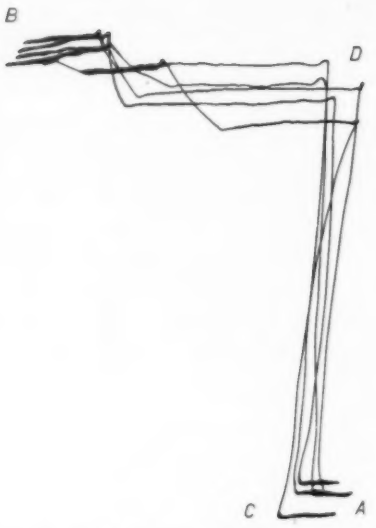

FIG. 1. Left eye divergence record on fixed photographic paper for transfer between fixed fixation points. Both fixation points lie in a sagittal plane. The more distant (B) lies above the nearer (A).

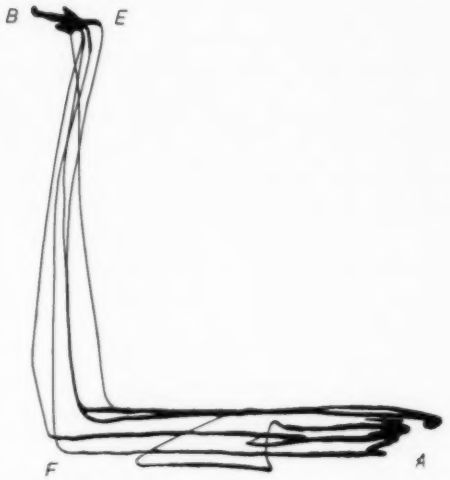

FIG. 2. Left eye convergence record on fixed photographic paper for transfer between fixed fixation points. Both fixation points lie in a sagittal plane. The more distant (B) lies above the nearer (A).

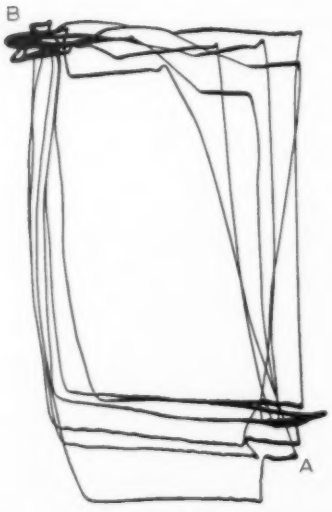

FIG. 3. Left eye record for alternating transfer between two fixation points lying in the sagittal plane. The more distant (B) lies above the nearer (A).

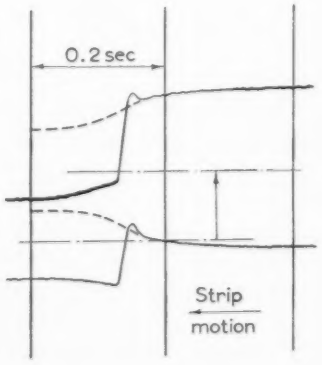

FIG. 4. Records for both eyes for fixation point transfer taken on a photokymograph.

(AC), the eye then performing a step in the direction of B (CD) followed by the main part of the divergence (DB). If the fixation points lie far apart and the step is not sufficiently accurate the second (DB) phase is often coupled to additional correction step movements. Fig. 1 shows these correction movements.

Fig. 2 shows a left-eye record for the B–A shift. Here a slight convergence occurs in the first period (BE), the step in the A direction being then effected (EF) followed by a fresh convergence (FA), also sometimes accompanied by correction movements.

Fig. 3 is a left-eye record for alternating A–B and B–A movements.

(2) A special device presented several fixation points lying at different distances and in different directions in a random order. In some experiments the subject was required to fixate a fresh point as soon as it appeared (unexpectedly), in others the subject was quite free to change the fixation point at will. Photokymograph records were made of the motions of both eyes. The records revealed that all such shifts between fixed points occurred as above, i.e. there was always a slight convergence or divergence prior to the step, followed by a second convergence or divergence phase afterwards.

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(3) A study of the various records showed that the convergence or divergence process in any fixation point shift is continuous no matter whether steps were present or absent (points on different or identical axes with respect to the mean eye axis respectively). In other words the divergence or convergence constitutes a continuous change in the distance of the fixation point at all stages. Any shift involving points not on the same axis involves two independent eye motions, i.e. convergence or divergence and a step.

When the fixation points lie on the same axis no step is required.

Fig. 4, taken for both eyes on oscillograph paper moving vertically, illustrates this. The arrow denotes the shift in the fixation axis (step). The dashed lines represent the divergence preceding the step displaced by the step height. It is clear that the separate sections of the divergence join together smoothly.

(4) In any shift between fixed fixation points the convergence (divergence) before the step lasts from 0·05 to 0·2 sec. Fig. 5 illustrates this. The upper section gives the duration (in seconds) of the whole process. Within each column the points denote the convergence duration for shifts between points lying at the following distances (in cm): 25→15, 35→25, 45→35, 55→45, 65→55, 75→65. The crosses denote the divergence duration in the 15→25, 25→35, etc., shifts. The lower section relates to the period before the step, the values being found from the records used for the upper section of the figure.

(5) Numerous such records were used to derive the scheme shown in Fig. 6. The left section illustrates the shift from a point A to a more distant point B. The right shows the reverse process. The first process consists of (*a*) divergence preceding the step over the section AC, the point of intersection of the eye axes moving along the mean eye line passing through A; (*b*) a step, i.e. an eye axis rotation towards B, divergence continuing; and (*c*) continued divergence, the intersection point moving along DB, i.e. the mean eye line directed towards B. The reverse process consists of (*a*) convergence preceding the step, the motion being along BE, i.e. the mean eye line directed away from B; (*b*) a step, i.e. eye axis rotation towards A, convergence

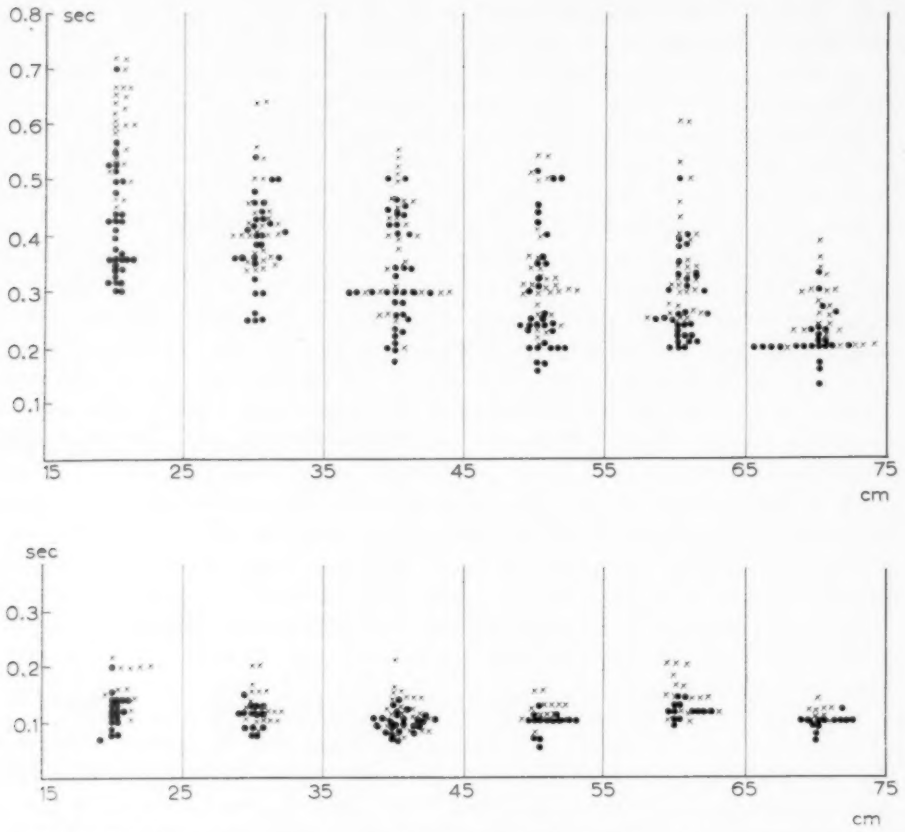

FIG. 5. Durations of eye convergence and divergence (top). Durations of eye convergence and divergence prior to the step (bottom).

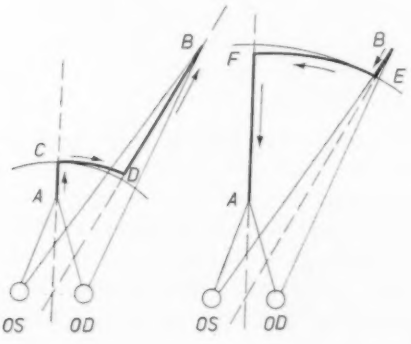

FIG. 6. Scheme of eye motion for transfer between two fixed fixation points.

continuing; and (*c*) continued convergence along FA, i.e. the mean eye axis directed towards A.

As we have already remarked, the fixation point shift is sometimes accompanied by additional correction steps. The eye motion is then still of the type shown in Fig. 6.

In many instances a slight divergence or convergence correction is applied at the end of the transfer. This effects no material change in the scheme shown in Fig. 6, but causes a considerable scatter in the convergence (divergence) duration, as is clear from the upper part of Fig. 5.

CONCLUSIONS

In any transfer between fixed fixation points which do not lie on the same mean eye axis the eye motion is compounded of two independent movements, i.e. convergence (divergence) and a stepwise movement. The convergence (divergence) always occurs in such a way that the point of intersection of the individual eye axes moves along the mean eye line, the direction of the latter changing stepwise. In any such transfer the stepwise motion is preceded by a slight convergence or divergence which is of about the same duration no matter how large the step or the separation in depth of the fixation points.

Translated by J. E. S. BRADLEY

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THE PERCEPTION OF AN IMAGE FIXED WITH RESPECT TO THE RETINA*

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(*Received* 6 *June* 1957)

THE fact that all visible differences in an image motionless with respect to the retina rapidly vanish has been dealt with in our two previous papers [1, 2]. These experiments showed that study of the perception of such images can give vital new information on the visual analyser function. It is therefore expedient to begin our study with a detailed exposition of the conditions under which these images cease to be perceived. The possible changes in the analyser produced by the light stimulus after all visible differences have vanished are of still greater interest. One of the primary problems is also, of course, the simultaneous perception of images fixed and in motion relative to the retina.

The methods have already been described in detail [1, 2]. Figs. 1 and 2 show only schematically the devices used to produce stationary retinal images. The caps have been improved by using very thin duralumin with crimped edges for the body, the crimping preventing the cap sliding about on the eye. Lenses of focal length 5 and

* Biofizika **2:** 6, 703–712, 1957 [Reprint Order No. BIO 93].

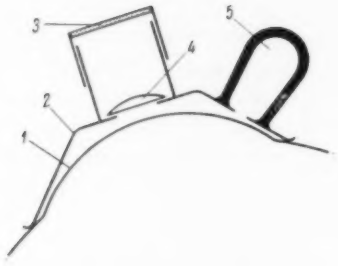

FIG. 1.

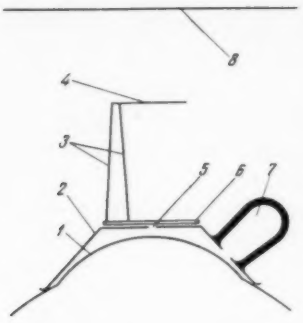

FIG. 2.

FIG. 1. The Type 1 cap, which gives a stationary retinal image over the whole field of view. (1) cornea, (2) body of cap, (3) opal glass, (4) lens, (5) rubber bulb. This bulb is used to reduce the air pressure in the space between cornea and cap. The atmospheric pressure causes the cap to adhere tightly to the eye and remain fixed relative to it (and hence to the retina). The lens enables the subject to see objects on the opal glass background clearly, the glass being illuminated by the coloured light in use in the test.

FIG. 2. The Type 2 cap, which gives a stationary retinal image over part of the field of view. (1) cornea, (2) body of cap, (3) steel braces for attaching the stop rigidly to the cap, (4) stop to shut off part of the field of view, (5) diaphragm, (6) glass plate, (7) rubber bulb, (8) background. Cap attached to the eye as in Fig. 1. The diaphragm enables the subject to see the stop clearly, the stop moving with the eye and thus remaining stationary relative to the retina, and also to see the background, which, though objectively motionless, moves relative to the retina.

8 mm were used. The weight of the whole device has been reduced to 0·1 g. The maximum brightness of the opal glass in the cap was 3000 apostilb.

To simplify the subsequent presentation the following points are to be noted.

(1) In most of the experiments the subject's other eye (i.e. that not used directly in the experiment) was covered by a black light-tight eyeshade, and will not be mentioned in the descriptions. If it is illuminated in any way this will be specially mentioned.

(2) The objects giving fixed retinal images will be termed the fixed test field. Those objects giving moving retinal images either on account of their own motion or that of the eye will be termed the moving test field. Radiations entering the subject's eye from the fixed test field will be termed stimuli fixed with respect to the retina, or fixed stimuli.

For instance, if a Type 1 cap (Fig. 1) is used, where a short-focus lens is fitted, the fixed test field will be the opal glass in the cap and all objects lying on it as background, the fixed test field also containing the dark surround to the opal glass. The moving test field can here be an object moving in front of the opal glass or the moving shadow of an object lying between the glass and the light source.

If the Type 2 cap (Fig. 2) is used (in which a diaphragm replaces the lens) the fixed test field will be a stop or stops rigidly fixed to the cap. The moving test field can be the whole of the real fixed background, which produces a moving retinal image because of eye motion. Also, as in the first type, the moving test field can be an object moving over the background of the stop.

(3) A fixed test field in which the subject has ceased to see any colour differences will be termed an empty field.

RESULTS

1

We carried out several series of experiments to establish as completely as possible the conditions under which all colour differences in the fixed test field vanished at constant illumination.

(1) The first question examined was whether an empty field always occurred if the colour brightness and angular dimensions of the various parts of the fixed test field were varied over the widest possible ranges. A Type 1 cap was used, this producing a fixed test field for the whole field of view. It was found that at fixed illumination the empty field always appeared after 1–3 sec and the field colour always became the same. The colour was usually termed dark grey, dark, darkish as with the eyes closed, etc., by the subject. If the other eye was illuminated (by covering with various colour filters which also diffused the light) the apparent colour of the empty field changed, acquiring a tinge the same as the colour admitted to the other eye.

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(2) We then did experiments in which one part of the field of view was occupied by the fixed test field and another by the moving one. The conditions for empty fields to appear and the colour changes seen were studied. A Type 2 cap was used with stops attached (fixed test fields) of various colours, variegations and sizes, while the backgrounds (i.e. moving test fields) on which the stops were seen were a large selection of variegated and single-colour paper sheets.

Just as in the previous experiments all visible colour differences vanished from the fixed test fields in 1–3 sec and a uniform empty field was seen. The colour of the moving test field remained unaltered. When the fixed test field was of smaller angular dimensions than the moving one, so that the former was seen as a whole on the latter, the colour of the empty field merged with that of the moving field and the empty field could not be perceived. In other words, if the stop was seen as a whole on the background of an evenly lit sheet of paper of uniform colour, the stop fused with the background after 1–3 sec, no matter what its size and colour, and was not seen (only the paper being seen). The stop here appears like the blind spot, which is also filled by the colour of the adjacent background.

If the moving field (background) was highly variegated (pieces of different coloured paper glued to cardboard) and the fixed stop was of angular dimensions considerably exceeding those of the uniform areas of the background (the paper scraps), the empty field could not fuse with the background and its apparent colour always remained dark grey and took on the hue presented to the other eye when this was illuminated.

When the fixed field and the separate uniformly coloured areas of the moving one were of about the same size, a consistent tendency for the colour of the fixed field to fuse with those of areas of the background was observed. Complete fusion occurred only if the empty field was seen as a whole against some part of the background of uniform colour.

(3) An experiment, illustrated by Fig. 3, was performed with the Type 2 cap. Two white fixed test fields *A* and *a* were placed in the field of view at some distance

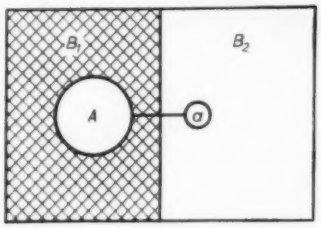

FIG. 3. A and a are white stops (test fields) fixed relative to the retina which the subject sees against the backgrounds B_1 and B_2, in motion relative to the retina. B_1 is variegated (a sheet of cardboard with scraps of coloured papers glued to it), B_2 being of a uniform colour (a sheet of red paper).

from one another, on the moving field backgrounds B_1 and B_2. The diameter of one (A) was 20°, of the other (a) 5°. The distance between the edges of the fields was 15°. The B_1 half of the background was variegated, with uniform areas of different colours and angular dimensions much less than 20°. The other half, B_2, was uniform in colour (a large sheet of paper). The subject's eye was set such that A was always seen against B_1 and a against B_2. We studied the colours of the empty fields when A could not fuse in colour with B_1 while a could with B_2. This amounts to determining whether the two empty fields could alter in colour independently. It was found that when the empty fields had appeared, A became dark grey in colour while the colour of a fused with that of B_2. When the other eye was illuminated, A, together with its background, changed in hue in line with the colour admitted to the other eye, while a was not seen. The result was unaffected if coloured or variegated stops were used instead of white for A and a.

(4) In a series of experiments using Types 1 and 2 caps, moving test fields of various colours and intensities were used against empty field backgrounds. The empty field colour was unaffected but small aureoles sometimes occurred round the moving fields. No differences in the fixed field were seen within the aureoles. This point is emphasized specially. The series of experiments is described in more detail below.

The data all show that, if the illumination is fixed, all colour differences in the fixed field cease to be seen after 1–3 sec and do not reappear under these conditions. This implies that the stimuli entering the eye from the empty field are not worked up into a visual image.

The apparent colour of the empty field is determined by the experimental conditions, and can alter. The perception of an empty field cannot be equated with the perception of the absence of light, i.e. that of a fixed black test field. A black colour corresponds to a signal that light is lacking, whereas an empty field gives no signal. The no-light signal causes us to see a black colour, whereas any colour can be seen in the empty field in the absence of a signal. It is clear that under normal conditions of perception we frequently encounter empty fields, as when one looks upon large surfaces uniform in colour, e.g. a cloudless blue sky or a uniform screen.

Although the human eye is always in motion its range in solid angle terms is frequently limited. If the uniform background is of larger angular dimensions than

this there must be an area on the retina on which no changes occur within the time required for the empty field effect (1–3 sec). An empty field must therefore arise in the corresponding area of the object, but this remains subjectively unnoticed because it takes the colour of its surroundings. In other words, it is as if we see the colour only at the edges of the uniform area, and extrapolate it to the centre.

II

An important topic in the perception of fixed test fields is the changes in the visual analysis system produced by the light after the empty field has appeared, i.e. after the unchanging stimuli cease to be worked into a visual image. The changes can be conveniently studied using after-images, which occur under normal conditions and depend on the light intensity and the time for which it acts on the retina. At this stage we record only that there are (or are not) such changes, using the differences between the after-images observed under defined experimental conditions.

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Only the first type of cap was used in these experiments. The opal glass was here covered by black paper with a rectangular hole in the centre, i.e. the fixed test field covered the whole retina and was a luminous rectangle on a black background (the rectangular opal glass in the cap). The rectangle was 20° high and 10° wide. The black background was kept unchanged. The rectangle was either left white or illuminated with various coloured lights using filters. The following Shott filters were used: red RG-1, blue BG-12, yellow GG-14, green VG-6 and purple UG-3.

(1) In a series of experiments in which all the above filters were used only the fixed field brightness was altered. The field colour and the exposure (1 min) were the same in any given experiment. The instant when the empty field appeared was reckoned as the zero of time (i.e. 1–3 sec after the start). We studied the way the after-images, seen on completely cutting off the light, varied with brightness alone. It was found that the colour and mode of extinction of the after-image varied widely with the fixed field brightness, from a colour the same as that of the test field (at high intensities) to one approximately complementary to it (at low intensities). The apparent colour of the empty field remained always the same.

In the next series the exposure alone was varied, the brightness being kept constant at a set value. The same after-images as before were obtained.

Further, when the fixed field was extremely bright (white, 3000 apostilb) and the exposure 3–5 min, a momentary flash (lasting about 0·1 sec) was seen by the subject on cutting off the light, followed by a very brief dark interval, giving way to the after-image as before. Here the after-image had the same colour as the test field immediately following the flash and darkness, while at the end it had, roughly speaking, the complementary colour.

(2) In subsequent experiments, in which filters were also used, the lower part of the test rectangle was covered with black paper to exclude the light before starting each test. Right at the start the subject saw a bright square on a black background (the upper part of the rectangle), the empty field appearing after 1–3 sec as before, the square fusing with the background. This state was maintained for 1–2 min. Then the black paper was carefully removed, so as not to disturb the fixed retinal image. The subject again saw a bright square for 1–3 sec, the square then fusing with the dark background, i.e. an empty field was produced with both halves equally

illuminated. After a further 2–3 sec the light was cut off completely, and two totally different after-images were seen. In particular, the brightness and exposure could be chosen such that the upper after-image had the same hue as the rectangle while the lower had the complementary one.

(3) In a series of experiments, illustrated by Fig. 4, the fixed field rectangle was fitted with a red filter and the lower half was also covered with a 15 per cent transmission neutral filter. At first the subject saw a red rectangle, the upper half being

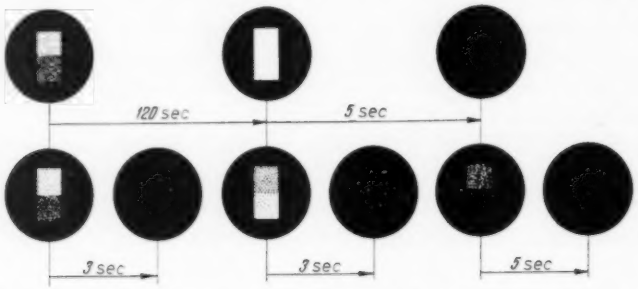

FIG. 4. The upper section shows the various fixed test fields, the lower the apparent field colours.

much brighter than the lower. After 1–3 sec the empty field was seen (both halves fusing with the dark background), this state being maintained for 2 min. The filters were then carefully removed together, so as not to disturb the fixed retinal image, and the rectangle was illuminated with direct white light. The rectangle was then seen as bright blue (i.e. close to the complementary colour) and as being divided into two very different sections. The upper, previously more brightly illuminated, half was bluer and darker than the lower. The empty field again appeared after 1–3 sec. The light was then cut off completely and the after-image appeared as a dark-blue rectangle, also consisting of two very different halves, but with the upper now brighter than the lower.

Analogous results were obtained with other filters. The extra colour seen was, roughly speaking, complementary to the filter colour.

The above data imply that the fixed unchanging stimuli which act after the empty field has appeared materially alter the analyser state. With identical stimuli and colours the state may differ widely, which appears as differences in the after-image. Two essentially distinct processes are at work, the first being a rapid one in which all visible differences in the fixed test field vanish, and the second a slower one, which is revealed by the after-image. While no more than 3 sec is needed for the empty field to appear when the stimuli are fixed in time and space, the changes in the analyser, as observed via the after-images, go on for some tens of seconds. The rapid process can be related to the electrophysiological finding of pulse trains in the visual nerve in response to intensity changes ("on" and "off" effects). The second, slower, process is related to adaptation changes.

III

The last topic to be considered here relates to the perception of objects moving over an empty field as background. Here, to facilitate exposition, we shall term "objects" those moving tests fields which move against an empty-field background.

(1) The fixed-field colour was changed widely, using a Type 1 cap, and when the empty field had appeared a black object 3° in diameter was moved across it. The empty field did not alter in colour, but a small aureole sometimes appeared round the object. The black object was seen as being of the after-image colour observed on switching off the light completely. This is to be expected, since the appearance of a black object in the field and cutting off the light reaching that area of the retina are processes which are in practice identical. It was also found that the apparent colour of the black object depended on its rate of motion as well as on the fixed-test-field colour. This occurs because the after-image colour varies in time, and the way the after-image appears and dies will depend on the way the object moves. In some instances this test gave rise to apparent bright coloured borders, of colour and brightness strongly dependent on the state of the eye and the nature of the motion.

(2) Then the tests were repeated with a bright white object 3° in diameter. The object brightness was always much higher than that of the fixed field. The apparent empty-field colour was not thereby altered but that of the object at first (i.e. just after the empty field appeared) was white and then (in 30–40 sec) changed appreciably, taking on a saturated hue approximately complementary to that of the fixed field.

(3) Tests were also done with a Type 1 cap in which the fixed field was bright white. A red moving object 3° in diameter was used, of brightness much below that of the fixed field. At the instant when the empty field appeared (i.e. the white became dark grey) the apparent colour of the red object became dark red, remaining appreciably darker than the empty field. The other eye was then illuminated with red light, the empty field thereby acquiring a red hue while the apparent colour of the object became more saturated, becoming "very red", but still, as before, darker than the fixed field. If blue light was used the empty field acquired a blue hue, the red object losing its apparent redness and abruptly becoming dark brownish, while as before remaining darker than the fixed field.

Although both background and object changed in apparent colour with the conditions, the difference between the two was always maintained.

(4) Fig. 5 illustrates one of the tests using a Type 2 cap. The moving field was a large sheet of red paper B illuminated by a table-lamp, a fixed field divided into two halves, A_1 and A_2 being kept on this background throughout the experiment. The fixed field was 20° in diameter. The left half A_1 was black, the right a bright white. The moving object was a small red circle C of the same colour as B. The diameter of C was 3°. The way the apparent colour of C varied as it moved over A_1 and A_2 was examined. We found that C took the same colour as B, the apparent colour of the empty field becoming under these conditions the same as that of B.

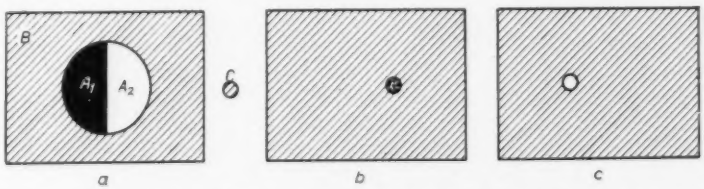

FIG. 5. A_1 and A_2 are halves of the fixed test field. B is the moving test field and C the object.

The moving object *C* was distinctly seen but the background itself (empty field) was not. In the right half of the empty field, *C* appeared a highly saturated red and darker than *B* (Fig. 5b). On the left it appeared a rose colour and lighter than *B* (Fig. 5c). Small aureoles were sometimes seen around it.

The apparent colour of a moving object on an empty-field background can vary widely with the conditions of the experiment and the state of the visual analyser, but the differences in brightness and colour between the two tend to be retained.

No moving test field can produce differences within the empty field. This shows that the processes producing the empty-field appearance are localized nearer the periphery of the retinal layers in which horizontal correlations are performed. Also, since the slow processes never cause any change in the apparent colour of an empty field they, too, are localized nearer the periphery than the ones which cause the empty-field effect.

CONCLUSIONS

(1) A test field fixed with respect to the retina in which all apparent colour differences have vanished is termed an empty field. The production of an empty field corresponds to a state in the visual analyser in which information from the outside world entering the human eye and producing unchanging stimuli fixed in position relative to the retina ceases to be worked into a visual image after 1–3 sec. The apparent colour of the empty field is determined by the experimental conditions but is quite independent of the radiation acting on the area corresponding to the empty field.

(2) The analyser state is materially altered by such unchanging positionally fixed stimuli continuing to act after the empty field has developed.

We observed two essentially distinct processes in the analyser, the first being fast, in which all visible differences in the fixed test field vanished, and the second slow, being detected via the after-images. The first process lasts less than 3 sec whereas the second takes tens of seconds.

(3) The apparent colour of a moving object on an empty-field background can vary widely with the experimental conditions and analyser state, but there is a tendency for the differences between object and empty field to be conserved.

In conclusion the author expresses his debt to the head of the Biophysics of Vision Laboratory, N. D. Nyuberg, Doctor of Physico-mathematical Sciences, for assistance in this work.

Translated by J. E. S. BRADLEY

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ACCURACY OF BRIGHTNESS CONTRAST MEASUREMENTS*

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(*Received* 7 *December* 1956)

WE have studied the accuracy of brightness contrast measurements made on instruments using extinction and photometric comparison. To this end we made a photometer apparatus in which any preset contrast could be produced with an accuracy much greater than that of any of the instruments under test. Fig. 1 shows the system used.

Beams from the source S (cineprojector lamp; 110 V, 300 W) fall on mirrors F_1 and F_2 and, after reflection from these, on the opal glasses N_1 and N_2. The comparison fields, which are observed via the lens O, are produced by the Lummer cube K, which lies at the focus of O. The instrument I under test is placed directly behind O. In the field of view of the instrument the observer sees the dividing line

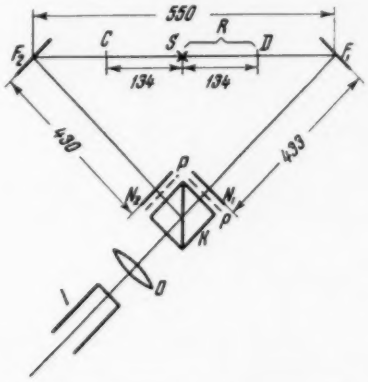

FIG. 1. The photometric apparatus. Dimensions in millimetres.

between the photometric fields produced by rays from F_1 and F_2. By displacing S along the F_1F_2 axis we can either produce a match between the fields or produce any preset contrast to be measured with the instruments under test. Tests showed that on average the device gave 2 per cent error in contrast measurements. This was quite satisfactory for our purpose. The brightness of the adaptation background was 1000–1500 apostilb.

In the work on accuracy of contrast measurement the instrument was set directly behind O. The observer saw a contrast produced either by displacing S from the match position along F_1F_2 or by inserting neutral filters in front of N_1 or N_2. Six contrasts were presented for measurement: 0·10; 0·15; 0·30; 0·40; 0·56; 0·81.

* Biofizika **2**: 6, 713–719, 1957 [Reprint Order No. BIO 94].

Table 1. Results for the IV-GGO

K— standard contrast	K—measured contrast													$\frac{\Delta K}{K}$
	1	2	3	1	2	3	1	2	3	1	2	3	Mean	
0·81	0·81	0·74	0·71	0·72	0·81	0·72	0·73	0·81	0·61	0·58	0·79	0·71	0·73	± 0·10
0·56	0·69	0·56	0·52	0·50	0·56	0·49	0·46	0·40	0·77	0·61	0·45	0·47	0·54	± 0·20
0·40	0·44	0·37	0·56	0·33	0·45	0·35	0·38	0·44	0·30	0·38	0·47	6·46	0·41	± 0·18
0·30	0·35	0·25	0·37	0·25	0·22	0·33	0·36	0·36	0·28	0·42	0·35	0·37	0·32	± 1·19
0·15	0·17	0·28	0·16	0·33	0·17	0·32	0·24	0·14	0·17	0·17	0·20	0·21	0·21	± 0·31
0·10	0·13	0·13	0·14	0·17	0·17	0·12	0·24	0·12	0·14	0·09	—	—	0·145	± 0·31

Table 2. Results for the pupil photometer

Standard contrast	Measured contrast													$\frac{\Delta K}{K}$
	1	2	3	1	2	3	1	2	3	1	2	3	Mean	
0·81	0·77	0·82	0·78	0·76	0·76	0·76	0·74	0·72	0·68	0·87	0·71	0·83	0·77	± 0·07
0·56	0·61	0·57	0·72	0·57	0·49	0·51	0·60	0·53	0·53	0·54	0·49	0·48	0·554	± 0·12
0·40	0·45	0·47	0·43	0·40	0·42	0·40	0,39	0·36	0·39	0·39	0·40	0·38	0·395	± 0·08
0·30	0·30	0·28	0·26	0·28	0·25	0·30	0·30	0·28	0·30	0·30	0·29	0·28	0·29	± 0·07
0·15	0·17	0·13	0·15	0·18	0·12	0·18	0·16	0·15	0·13	0·12	0·14	0·13	0·147	± 0·15
0·10	0·10	0·12	0·10	0·11	0·12	0·14	—	—	—	—	—	—	0·115	± 0·12

An IV-GGO visibility meter was used as typical of extinction instruments [1]. Four series of observations were made with this apparatus. Three observers experienced in using the IV-GGO were used in each series. Each observer measured the six contrasts above. When an unknown contrast was to be measured each observer made seven readings relative to an absolutely black test-field (a screen being placed between the Lummer cube and opal glass to prevent light reaching one half of the field of view) immediately followed by seven on the unknown. The first two readings on the absolute black and on the unknown were discarded as preliminary adjustment is involved. The observers were unaware of the readings they made. Table 1 gives the measurements made on the IV-GGO. Each figure is the mean of five observations for absolute black and five for the unknown for each observer (seven observations being made, the first two being discarded).

The last column in Table 1 gives the root mean square error of constrast measurement, $\Delta K/K$, for a single observation deriving from the two sets of five observations above. Table 1 shows that the contrast values obtained for a fixed contrast by different observers at one time or by one observer at different times may differ by a factor of 2. But the means of twelve separate observations by different observers are very close to the true value, and $\Delta K/K$ (derived as above) was ± 20 per cent.

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Thus, the $\Delta K/K$ are the same, at ± 20 per cent, for one of the early prototypes of the GGO-DM-7 haze meter [2] and for visibility meters of other designs [1]. In field conditions the haze-meter readings showed a relative error of contrast measurement of ± 20 per cent [3]. This indicates that the error of contrast measurement using extinction methods is independent of the apparatus design and is determined, as stated above, by the eye, i.e. by the stability of the contrast threshold [3, 4].

Our data of 1948 [5] show that $\Delta\varepsilon/\varepsilon$, the stability of the contrast threshold ε, does not exceed 0·20. This agrees with Blackwell's [6] data (he gives a probability distribution for the contrast threshold) and with Siedentopf's [7], the latter giving $\Delta\varepsilon/\varepsilon = 0{\cdot}3$ for one observer and 0·45 for the divergence between several observers. Hence, the cause of the large errors ($\Delta K/K \pm 20$ per cent) in measuring contrasts of objects against a background using haze meters should be sought not in bad equipment design but in the eye, which has an unstable contrast threshold.

Since contrast measurement by extinction, i.e. in units of contrast sensitivity threshold, which varies randomly, is unsatisfactory, contrast measurement by photometry is more reliable as the contrast sensitivity threshold is not the unit but the error of measurement. We examined photometers in which equal contrast of two objects on a background, and not the vanishing of a field boundary, was the operative principle. This is done in Sharonov's diaphanoscope [8] and in Boldyrev's pupil photometer. This latter (Fig. 2) is composed of a sight tube within which the photometer device is set up near the real image of the pupil, the focal plane of the lens 1 containing the strip 2 which produces the comparison contrast. The images of 2 (formed by the lenses of the rotating system 3) and of the remote object used are seen equally sharply via the eyepiece 4. The photometer device consists of the diaphragm 5 with two rhombohedral holes, one hole being overlapped by the optical wedge 6, which deviates the rays through a certain angle. The diaphragm 5 is overlapped by the moveable slide 7 with a cut-out angle of 120°; this can be used to vary the area of one hole. The wedge splits the images into two. One is visible through the hole

covered by the wedge and is therefore displaced relative to the other, seen through the free hole. The image of the opaque strip *a* is also doubled.

When measuring the contrast of some distant object against the sky the observer compares the displaced image of the object seen through the wedge with the undisplaced image of the strip. The image contrasts are altered by adjusting the area of one hole. The contrast of the displaced image will, for instance, be increased while that of the undisplaced one will be reduced. By comparing the contrast of the undisplaced image from the strip with that of the displaced image from the object the two can be matched. It is readily seen that when the strip is opaque the contrast to be measured equals the ratio of the hole areas.

In all, four series of observations were made on this photometer, with three observers in each series, each measuring the six contrasts above. On each contrast

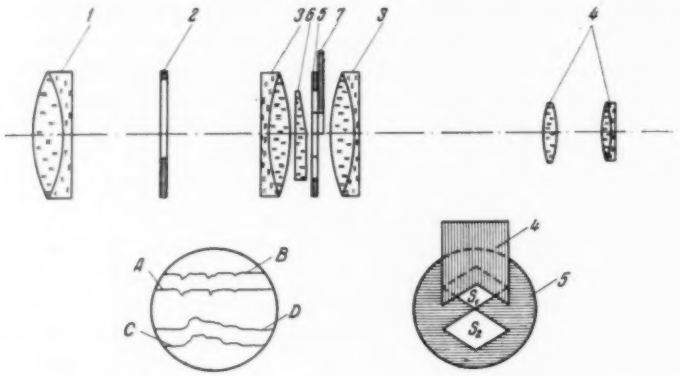

FIG. 2. The pupil photometer. (*A*) undisplaced image of strip, (*B*) displaced image of strip, (*C*) undisplaced image of object, (*D*) displaced image of object.

five readings were made, the observer being unaware of the readings made. Table 2 gives these results. Each figure is the mean of the five readings for one observer. The last column gives $\Delta K/K$ computed from five readings, this being on average 10 per cent. The maximum difference between readings for a given contrast was on average 30 per cent. This error is found only if the object and strip show only brightness contrast at the match point. This can always be ensured by designing the instrument to apply as it were a very dense haze, to obliterate all colour differences, and this should always be borne in mind in designing such equipment.

We shall show that the errors of measurement in photometric comparison depend greatly on whether the strip and object are similar in size, shape and colour. The more similar they are, the less the error. Thus, stripes of various angular sizes and shapes should be provided.

Using the standard arrangement we studied the accuracy of contrast measurements on Sharonov's diaphanoscope [8]. Here a large image occupying half the field of view is compared for brightness with a very small square dimmer in the diaphanoscope. The setting-up and testing were as previously, three observers being used; as the scale is stepped the observers gave only one reading.

Table 3 gives the results for four contrasts, each figure being the one reading from one observer. The last column gives $\Delta K/K$ for one observer.

TABLE 3. CONTRAST MEASUREMENTS ON THE DIAPHANOSCOPE

Standard contrast	Measured contrast							$\frac{\Delta K}{K}$
	1	2	3	1	2	3	Mean	
0·56	0·29	0·74	0·86	0·29	0·80	0·29	0·54	± 0·46
0·40	0·57	0·48	0·29	0·72	0·21	0·21	0·41	± 0·48
0·30	0·14	0·48	0·57	0·14	0·21	0·21	0·29	± 0·57
0·15	0·29	0·08	0·08	0·29	0·14	0·21	0·18	± 0·53

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It is seen that, on average, $\Delta K/K$ is ± 50 per cent. To determine the least possible error when a photometric match is obtained with contrast equality for two objects on a background, and not from a dividing line becoming invisible, we carried out the following experiment. Three observers were required to match in brightness a displaced and an undisplaced image a certain distance apart on a sky background in the haze meter. Here the objects compared are exactly the same. The accuracy was ± 5 per cent, and this may be considered as the limiting accuracy for such photometers.

We then determined the accuracy of visual contrast estimates for an object on a background using the standard contrasts [5, 9]. Three observers were required to set contrasts of 0·15, 0·30 and 0·50 by moving S along F_1F_2. Each observer was familiarized with these contrasts before the tests.

Table 4 gives the results. Each figure results from one setting by one observer. Here $\Delta K/K = 30$ per cent. Thus, visual estimates without instruments are of accuracy comparable with instrumental readings. Lazarev found the same result [10].

TABLE 4. ACCURACY OF VISUAL CONTRAST ESTIMATES

$K = 0{\cdot}15$			$K = 0{\cdot}30$			$K = 0{\cdot}50$		
1	2	3	1	2	3	1	2	3
0·10	0·08	0·19	0·34	0·42	0·40	0·44	0·43	0·72
0·10	0·11	0·21	0·26	0·38	0·38	0·62	0·42	0·35
0·09	0·19	0·09	0·28	0·40	0·22	0·58	0·64	0·65
0·11	0·19	0·09	0·26	0·24	0·42	0·44	0·40	0·67
0·17	0·13	0·20	0·22	0·24	0·23	0·30	0·58	0·70
0·09	0·11	0·19	0·26	0·34	0·20	0·35	0·58	0·36
0·12	0·10	0·19	0·42	0·21	0·21	0·58	0·62	0·40
0·12	0·11	0·11	0·25	0·22	0·37	0·72	0·55	0·50
0·16	0·10	0·10	0·40	0·39	0·21	0·42	0·43	0·62
0·18	0·16	0·10	0·40	0·22	0·39	0·59	0·55	0·58
Mean								
0·12	0·13	0·15	0·31	0·31	0·30	0·50	0·52	0·56
$\frac{\Delta K}{K} = \pm 0{\cdot}27$	± 0·28	± 0·33	± 0·24	± 0·27	± 0·30	± 0·26	±0·18	± 0·29

Let us now consider an example of the use of these data on the accuracy of brightness contrast estimates in practice. In meteorology the atmospheric transparency, or meteorological visibility range (MVR), is measured from the fall-off in contrast with distance. Here the mean square error of measurement of the MVR, $\Delta S/S$, takes [4, 5, 9] the form:

$$\frac{\Delta S}{S} = -\frac{S}{l \ln \varepsilon} \sqrt{\left(\frac{\Delta K_0}{K_0}\right)^2 + \left(\frac{\Delta K}{K}\right)^2} \tag{1}$$

where S is the MVR, l the distance to the object observed, ε the contrast sensitivity threshold, 0·05, $\Delta K_0/K_0$ the error of measurement of the true contrast against the background and $\Delta K/K$ the error in the contrast as weakened by the atmospheric haze layer l.

Equation (1) shows that $\Delta S/S$ depends solely on the accuracies with which K and K_0 are measured, at a given S/l. Assuming $\Delta K_0/K_0 = \pm 0{\cdot}15$ (no snow) and $\pm 0{\cdot}30$ (snow covering) [5, 9] for all devices, and taking $\Delta K/K$ from our data above, (1) gives a theoretical value for the error of measurement of the MVR which is a limit to the accuracy of any instrument.

TABLE 5. ACCURACIES OF VISUAL AND INSTRUMENTAL MVR MEASUREMENTS

Observation condition	$\frac{\Delta S}{S}$				
	Photometers extinction—smoke measure $\frac{\Delta K}{K} = \pm 0{\cdot}20$	Ideal photometers $\frac{\Delta K}{K} = \pm 0{\cdot}05$	Pupil photometer $\frac{\Delta K}{K} = \pm 0{\cdot}10$	Diaphanoscope $\frac{\Delta K}{K} = \pm 0{\cdot}50$	Visual evaluation with standard contrasts $\frac{\Delta K}{K} = \pm 0{\cdot}30$
No snow cover $\frac{\Delta K_0}{K_0} = \pm 0{\cdot}15$	$0{\cdot}083\frac{S}{l}$	$0{\cdot}053\frac{S}{l}$	$0{\cdot}060\frac{S}{l}$	$0{\cdot}174\frac{S}{l}$	$0{\cdot}112\frac{S}{l}$
Snow cover $\frac{\Delta K_0}{K_0} = \pm 0{\cdot}30$	$0{\cdot}12\frac{S}{l}$	$0{\cdot}100\frac{S}{l}$	$0{\cdot}103\frac{S}{l}$	$0{\cdot}19\frac{S}{l}$	$0{\cdot}14\frac{S}{l}$

Instrumental MVR measurements from the contrasts of distant objects are of accuracy comparable with visual estimates (Table 5). There is therefore no sense in determining K instrumentally with very high accuracy at the instant of observation.

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ACTION OF BLINDING INTENSITIES ON RETINAL FUNCTION IN THE FROG*

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VOL. 2 1957

(*Recieved* 9 *December* 1957)

THE fireball from a nuclear explosion can damage the eyes. Blinding occurred at Hiroshima and Nagasaki, with members of the U.S. Air Force during nuclear weapons tests, and with experimental animals placed at various distances from the epicentre [1–4]. Burns to retina and vascular envelope occurred up to 68 km away from the explosions.

Several workers [3–5] have claimed that the blinding is occasioned by the visible, infra-red and ultra-violet components, much accentuated by the focussing by the refracting media in the eye. The factors which influence the degree and extent of the damage to the fundus are the explosion yield, the fireball temperature, the distance from the epicentre, the state of the atmosphere, the pupil size (some 64 times smaller by day than by night) and certain other factors difficult to take into account [1–5].

In our experiments we tried to reproduce the flash of a nuclear explosion. V. M. Anan'ev constructed a powerful flash lamp of almost point dimensions (discharge gap less than 5 mm). The lamp was fed from a 300 μF capacitor charged at 3 kV. The discharge duration was <3 msec. Changes in the fundus observable ophthalmoscopically, and similar to those found after nuclear explosions, were thus produced with rabbits.

In this work 26 isolated eyes from frogs (*R. radibunda*) were used, the front section together with all refracting media being removed from 15.† Electroretinography was used to determine functional states. The biopotentials were picked up with silver chloride electrodes, one being fastened to the cornea or directly to the retina, the other to the point where the visual nerve emerges. A five-stage a.c. amplifier feeding a 9-pen Siemens oscillograph was used for recording. The light from a 30 W bulb at 1 m from the eye was found adequate to produce electroretinograms in our experiments.

Before the blinding flashes 3–5 recordings were taken, followed by ones 2 min afterwards and thereafter at 5–10 min intervals for 1–2 hr.‡

† This was done to elucidate the role of the focussing properties of the refracting media when high-intensity flashes were used.

‡ Further observations were useless as the preparation degenerated.

* Biofizika **2:** 6, 720–723, 1957 [Reprint Order No. BIO 95].

The results were the same with intact eyes and with ones with the refracting media removed. No distinction could be made between them from the electroretinograms.

Before blinding the curves had a certain specific shape (Fig. 1a).

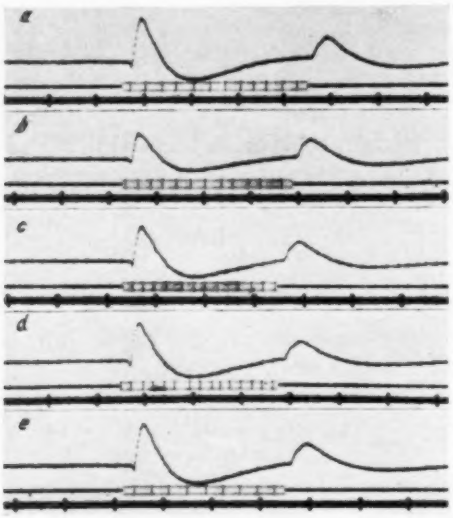

FIG. 1. Changes in frog electroretinograms caused by high-intensity light flashes (experiment no. 3). Top to bottom: (a) before flash, (b) 5 min after flash, (c) 10 min after, (d) 25 min after, (e) 45 min after. Light stimulus and time marks shown on the records.

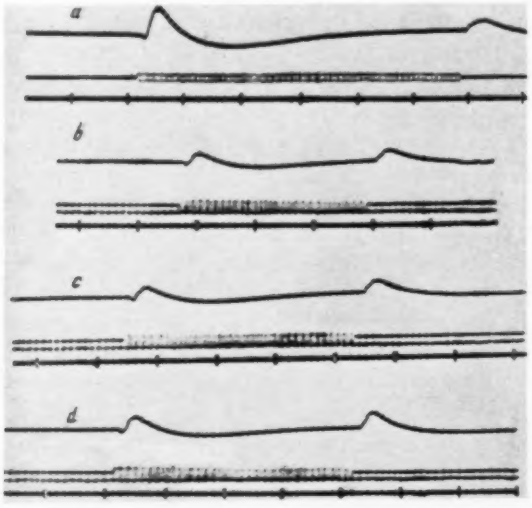

FIG. 2. Electroretinogram changes produced when high-intensity light flashes act on frogs' eyes (experiment no. 7). (a) before flash, (b) 5 min after, (c) 10 min after, (d) 1 hr 30 min after.

The retinal response was much weaker for 10–30 min after the flash, all elements, particularly the *c*-wave, being depressed (see Figs. 1 and 2). In 14 cases the curves approached normal in 20–60 min after the flash (Fig. 1) and in 12 they remained depressed right up to the end (Fig. 2). The depression is direct evidence that very bright flashes affect the retina strongly. But the fact that the e.r.g. approached the original level after 20–60 min in some cases does not indicate that the retinal function is fully restored. Our experiments show that retinal functions remain affected.

On current electrophysiological ideas the above e.r.g. changes indicate a shift in the trophic state of the retina and disturbances in the pulse trains passing from the retina to the higher levels of the visual analyser. The functional and morphological changes in the retina produced by blinding may to some extent be compensated, as the power of recovery of the e.r.g. even at 20–60 min after the flash indicates. The fact that no differences in the e.r.g. changes are visible as between intact eyes and ones from which the refracting media have been removed indicates that the focussing effects of the refracting media in the eye do not here play a decisive part.

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HUMAN ELECTROENCEPHALOGRAPHY DURING STIMULATION OF THE EYES BY RECTANGULAR PULSES*

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(*Received* 1 *July* 1957)

IN studying the lability of the visual analyser using a super-threshold intermittent current, the hypothesis has been advanced (Semenovskaya 1948–53) that the results obtained should be referred to the higher divisions of the visual analyser. The bases for this hypothesis are:

(1) Lability is characterized by a critical frequency of disappearance of phosphene in individual subjects. Such an analysing act could only be effected by the obligatory participation of the cerebral cortex, as with every act of consciousness.

(2) In developmental defects localized in the cortex—for example, in congenital colour blindness—the critical frequency is sharply reduced.

* Biofizika **2:** 6, 724–733, 1957 [Reprint Order No. BIO 96].

(3) The critical frequency is also reduced in periods of sleep when diffuse cortical inhibition occurs.

So far only very limited research has been conducted on animals by stimulating the cortex with an electric current and simultaneously recording the electrical potentials of the cortex. The first to do this was Danilevskii (1891) followed by a number of other workers [1–6]. Recording of the electrical potentials of the cortex on stimulation of the optic nerve by an electric current [7–10] has also been achieved.

Chang and Kaada [9] stimulated the exposed optic nerve of a cat under nembutal anaesthesia by discharges through a thyratron and led off the cortical currents from the gyrus lateralis. A response was obtained in the form of six deflexions of the peak type and a slow wave. The authors attribute these waves to the responses of those layers of the sub-cortex and cortex through which the exciting stimulus runs. Bishop and Clare [10] explain the potentials of differing polarity obtained from the surface of the cortex in response to stimulation of the afferent system as responses of the excited neurone elements in the different cortical layers.

To our knowledge no direct recording has been made of the electrical activity of human cortical cells upon stimulation of the eyes by an intermittent current. This paper describes the solution to this technical problem proposed by Zhdanov.

TECHNICAL CONDITIONS AND METHODS

An electrical device was constructed, the plan of which is presented in Fig. 1.

The channel for recording the electrical signals of stimulation consisted of a one-stage symmetrical amplifier, the input of which was connected to the primary winding of the impulse transformer and the output to a loop oscillograph.

As the source of sound, in those experiments in which sound was used as a conditioned stimulus, we used an EG-10 generator. The audio-frequency voltage from the output of the generator was fed simultaneously through a double-pole switch K_2 to a loudspeaker and to the recording loop of sound stimulation. The loudspeaker was placed 1·3 m in front of the head of the subject who was in a screened chamber. For the e.e.g. recordings a six-channel amplifier with full feeding from an alternating current grid was employed.

The channel of the response of the subject to stimulation, which was occasionally brought into use, consisted of the switch K_3, battery B_2 and the oscillograph circuit.

The recordings were made on standard moving film by means of an eight-channel oscillograph MPO-2.

The generator of rectangular pulses, the EG-10 sound generator, the amplifier of the e.e.g. channels, the accumulator battery B_1, and the control panel with the switches K_1 and K_2, were located on the bench near the oscillograph within reach of the experimenter.

The introduction of an impulse transformer and use of an autonomous amplifier to record the electrical signals of stimulation largely eliminated the parasitic inductions inherent in such systems. The main task was to separate the stimulator circuit from that of the subject in such a way that between them magnetic, but not electrical, connexions existed. The impulse transformer was chosen with a view to maintaining the rectangular form of stimulus. The rational choice of earthing for the individual nodes of the device also contributed to reducing the level of induced potentials. In

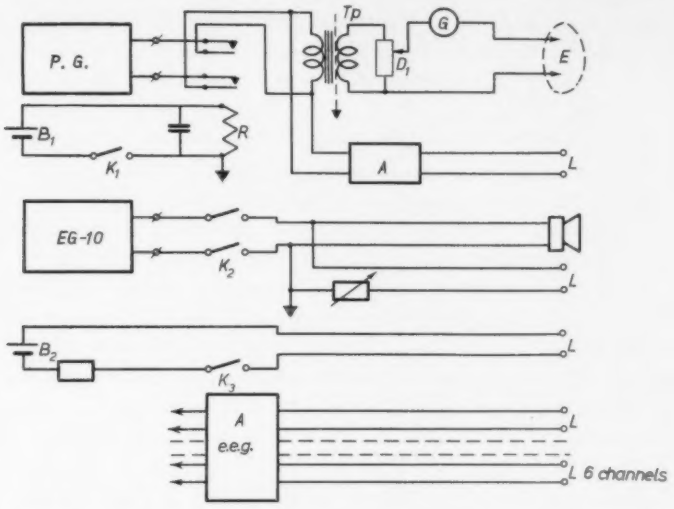

FIG. 1. Electrical arrangement. *PG*—pulse generator; *T*—impulse transformer; *R*—cut-in relay of pulse generator, fed from battery B_1 (relay circuit closed by switch K_1); D_1—divider of voltage, voltage from which is fed through galvanometer *G* to stimulating electrodes *E*; *L*—loops; *A*—amplifier.

order to cut down direct penetration of the signals of stimulation in the e.e.g. channels the method of localization of the points of electrical stimulation was employed. For this purpose these points (the eyes) were surrounded by a closed, current-conducting, flexible circuit.

In the present communication it is our intention to present e.e.g. recordings made during stimulation of human eyes by an intermittent current of 10 msec duration, i.e. the current which we chiefly employ in routine experiments on the lability of the visual analyser as indicated by the critical frequency of disappearance of phosphene.

In some cases we tested stimuli of 2–5 msec and 14–20 msec. The frequency of the stimulation varied within limits of 10–20–48 c/s. The voltage, measured with

FIG. 2. Arrangement of electrodes. 1—lead electrodes; 2—stimulating electrodes; 3—moist ring around eyes in the form of a closed current-conducting circuit.

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determination of the rheobase, was applied at sub-threshold, threshold and super-threshold strengths but did not exceed 6–7 V at the output of the stimulator. Hence in our tests the conditions of stimulation and lead-off considerably differed from those usually applied in animals, where, as for example, in the work of Roitbak [3], the duration of the stimulus was 0·2–0·5 msec and the voltage 30 V, while the distance between the lead-off and stimulating electrodes on the brain of a cat was 1·5 mm. In our experiments this distance was many times greater.

In some experiments a conditioned reflex was elaborated by combining a sound of specific current and intensity with an intermittent current.

In order to stimulate the eyes we used eye frames to which silver electrodes were attached by means of moveable hinges, making it possible to arrange the silver rods of the electrodes in any desired way. The tips of the rods were wound with cotton wool moistened with normal saline. Usually we placed the electrodes on the eyelids of both eyes. Fig. 2 shows the arrangement of the electrodes on the eyes of the subject. The experiments were performed on four normal subjects aged between 19 and 55.

We give below some of the most interesting features of 247 e.e.g. recordings made during the course of 15 experiments.

EXPERIMENTAL FINDINGS

The basic fact was established that, in the conditions of our experiments, it was possible to obtain e.e.g. recordings on stimulation of the eyes by an intermittent current.

Fig. 3 shows the e.e.g. recordings of the left parieto-occipital region of one subject: the recordings were made with the film moving at different speeds. In recording *a* the duration of stimulus was 10 msec, i.e. the period at which we observed the highest critical frequency of disappearance of phosphene.

It is clear from the records (Figs. 3 *a* and *b*) that when the band was moving at 250 mm/sec the electrical stimulus caused a make-and-break peak between which a slow cortical response wave was noted equal in duration to that of the stimulus. With the duration of stimulus unchanged at 10 msec a change in frequency from 40 to 28 c/s caused a change in frequency of response (Fig. 3 *a*). When the duration of stimulus was doubled it gave rise to a wave twice as long in the e.e.g. tracing, equal to 20–15 msec (Fig. 3 *b*).

When the band was moving more slowly (100 mm/sec) and the duration of the stimulus changed from 3 msec to 20 msec (Fig. 3 *c*), it was clear that the very short stimuli caused only a rise of the e.e.g. tracing at the break; but that as the stimulus was lengthened, the slow splash of cortical potential between the make and break became ever clearer.

Fig. 3 *d* shows that as the strength of current rises so does the amplitude of the e.e.g. response wave to each stimulus. Fig. 3 *e* and *f* against an attenuated background shows the variations in cortical electrical activity with a rate of electrical stimulation of 17 c/s.

The small amplitude of the slow response wave of the cortical cells (in comparison with the amplitude obtained in animal experiments) [3, 9, 10] is to be explained by the fact that the voltage was 4–5 times less and the distance between the stimulating

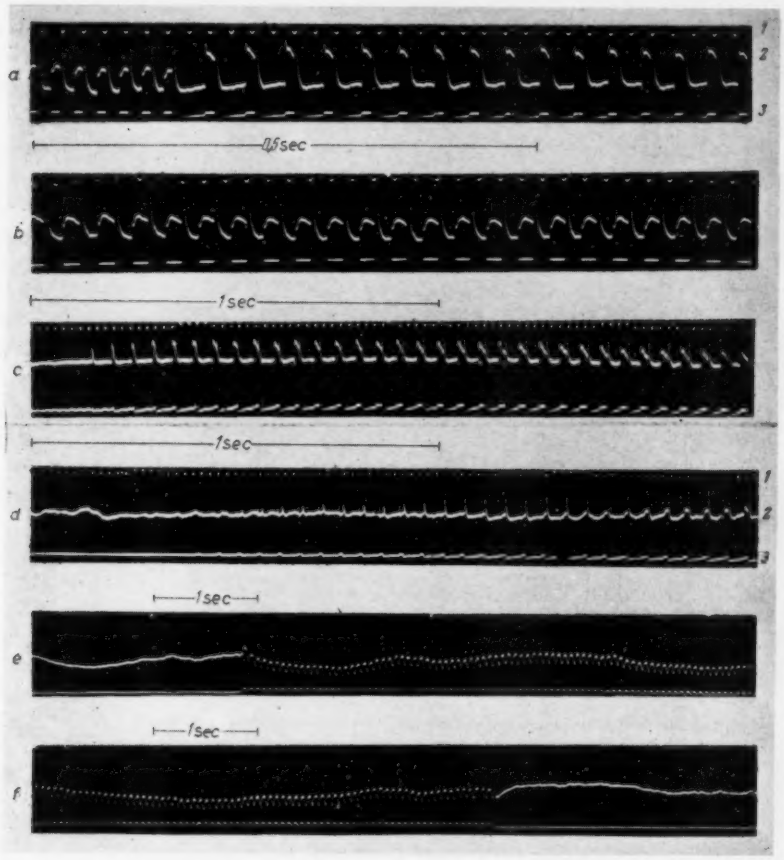

FIG. 3. Subject S, 17 June 1954. Cortical response of left parieto-occipital region of human brain to stimulation of eyes by rectangular pulses.
(*a*) Stimulation frequency of 40 c/s passing to 28 c/s, duration of stimulus remaining constant at 10 msec. Current—20 mA, twice threshold. 1—time 50 c/s; 2—cortical response; between two peaks at switch-on and switch-off of stimulus—slow wave of cortical potential; 3—electrical stimulation of eye.
(*b*) Order of recordings as in (*a*). Stimulation frequency 25 c/s, duration of stimulus—20–15 msec.
(*c*) 1—time; 2—cortical response: between two peaks at switch-on and switch-off of stimulus—slow wave falling off in amplitude; 3—stimulus gradually increased from 1 msec to 20 msec, frequency of stimulation 21 c/s.
(*d*) 3—gradual increase from sub-threshold level of current; gradual adjustment of rhythm of cortex to that of stimuli; 1—time.
(*e*) Subject S, 22 June 1954. Reproduction by cortex of rhythm of stimulation of 17 c/s, duration of stimulus 22 msec, order of recordings as before.
(*f*) Continuation.

(on eyes in man) and lead-off (over occipital or frontal cortical region) electrodes was 100 times greater.

Later we began to use a combination of a sound of a definite characteristic with a 20 and 10 c/s intermittent electric stimulus. Vivid and complicated transformations of cortical rhythms were obtained, i.e. changed lability of the cortical cells.

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In Fig. 4 *a*, subject X (25 May 1956), against a background of attenuated electrical activity, we see a reproduction of the rhythm of the 21 c/s electric stimuli in the frontal and occipital regions, worse in the left occipital region with consequent after-effect.

In the experiment of 7 June 1956 on subject X, against a background of slow cortical rhythms of 6 c/s, electric stimuli of 10 c/s (Fig. 4 *b*) produced in three e.e.g. leads a large amplitude rhythm of 10 c/s (sub-threshold voltage, approx. 1 V; threshold in subject X 1·5 V). On switch-off of current the 10 c/s rhythm disappeared from the e.e.g.; in the e.e.g. artifact peaks can be seen in the electrical stimulation.

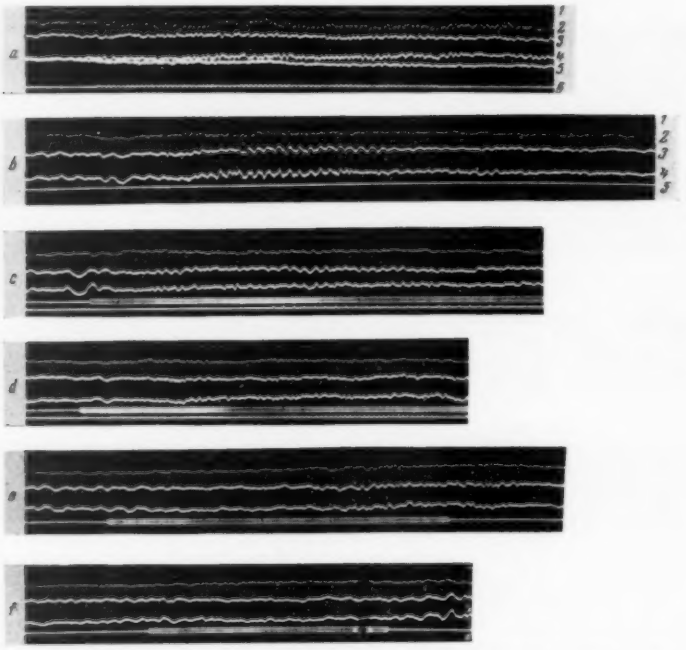

FIG. 4. Reproduction by cortex of rhythm of electrical stimulation with application of sound as a conditioned stimulus. Subject Kh., 18 years old.

(*a*) 8 June 1956. 1—time; 2—e.e.g. of right occipital region: reproduction of rhythm of 21 c/s; 3—e.e.g. of right frontal region; 4—e.e.g. of left occipital region; 5—e.e.g. of left frontal region; 6—intermittent electrical stimulation of 21 c/s, 5 V, duration of stimulus 14 msec.

(*b*) 7 June 1956. Leads: 2—from right and left frontal regions; 3—from right parieto-occipital region; 4—from left parieto-occipital; 5—intermittent current, 1 V, 10 c/s, duration of stimulus 10 msec (sub-threshold). Intensification of α-rhythm of stimulation against background of slow activity, on withdrawal of 10 c/s current, rhythm or 10 c/s disappears from cortex.

(*c*) Leads from cortex same as in (*b*). Sound of 250 c/s, 60 dB over auditory threshold is combined for second time with current, 5 V, 10 c/s, duration of stimulus 10 msec.

(*d*) Same leads. Sound of 400 c/s, 60 dB is combined with current of 20 c/s. Against background of slow activity sound alone produced in cortex a rhythm of 16–20 c/s and sound reinforced after 2 sec with a current reproduced a rhythm of 20 c/s.

(*e*) Leads same. Sound alone after an interval of 2 sec produces a rhythm of 16–20 c/s. Inhibition in after-effect.

(*f*) Leads same. Reflex to sound as conditioned stimulus fades away.

Against a background of slow waves, a sound of 250 c/s, 60 dB above the auditory threshold, which had earlier during the course of three days been combined 47 times with a 20 c/s current, produced a rhythm of 16–20 c/s in three leads—frontal and right and left parieto-occipital. When the 10 c/s current was again switched on, the 20 c/s rhythm in the cortex disappeared and was replaced by a rhythm of 10 c/s (Fig. 4 *c*).

A sound (400 c/s, 60 dB above auditory threshold) was combined 9 times with a 20 c/s current. A rhythm of 16–20 c/s was induced in the cortex by the sound alone and by the sound plus current (Fig. 4 *d*).

In Fig. 4 *e*, against a background of slow electrical activity, sound alone within 2 sec (the interval in the previous experiments was equal to the period of action of the sound before switching on the current (Fig. 4 *d*)) reproduced a rhythm of 20 c/s of a conditioned reflex nature with periods of inhibition (fluctuation). On withdrawal of the sound within 0·5 sec of the after-effect, inhibition of electrical activity was observed.

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Fig. 4 *f* shows the dying away of the conditioned reflex—a sound of 400 c/s, 60 dB, did not produce a rhythm of 20 c/s and only slightly arrested the slow activity of the cortex. Reproduction of the rhythm of stimulation in the cortex by a conditioned reflex is a difficult and quickly fading process.

SUMMARY AND CONCLUSIONS

(1) E.E.G. recordings were made in man during stimulation of the eyes by rectangular pulses from a generator fed from an alternating current grid. Recordings were possible without alternating current inductions.

(2) A clearly marked transformation of rhythms occurred in the cortex upon stimulation with a series of rectangular pulses at frequencies of 10–15–20–48 c/s when the duration of the stimulus was 5–10–20 msec. Transformations were not seen in the cortex when the duration of stimulus was 2–4 msec. A sub-threshold direct current voltage (in our case 1 V) sufficed to produce transformations in cortical rhythms, as compared with the pre-existing e.e.g. background.

(3) The effects of the frequencies under test were spread beyond the optical region despite the fact that the stimuli were applied to the eyes.

(4) After a specific and characteristic sound had been combined 10–20 times with an intermittent current, the sound produced in the cortex a conditioned reflex transformation of rhythms similar to that produced by the intermittent current.

(5) In the period of the elaboration of the conditioned reflex, the cortical background was often seen as small 6 c/s waves (retardation of cortical rhythms during elaboration of a conditioned reflex as observed by Peimer and Fadeeva and others [11]).

(6) When the action of the conditioned stimulus was not reinforced, slow rhythms or low activity appeared in the e.e.g.

(7) An after-effect was often noted in the rhythms induced by an intermittent current after switch-off of the current.

(8) All the findings described above indicate that, in determinations of the lability of the visual analyser by means of rectangular pulses, the higher cortical centres are involved.

(9) The theory of I. P. Pavlov that the visual and other analysers are widely represented in the cortex is confirmed.

Translated by A. CROZY

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A NEW ANOMALOSCOPE*

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(*Received* 20 *June* 1956)

(1) THE new anomaloscope was designed assuming colour to be three-dimensional; this assumption is at the root of all precision colorimeter measurement. The experimental fact of colour being three-dimensional is explained physiologically by there being three systems of light-sensitive receptors in the retina. The effects of any given radiant flux on these three systems of receptors give the three fundamental parameters of the radiation which determine the colour it has for us.

(2) As it has been established that the effects of radiant energy on the retina are additive we can write the following three expressions:

$$\int E(\lambda) \cdot \rho(\lambda) \cdot d\lambda = \bar{\rho}, \quad \int E(\lambda) \cdot \gamma(\lambda) \cdot d\lambda = \bar{\gamma}, \quad \int E(\lambda) \cdot \beta(\lambda) d\lambda = \bar{\beta}.$$

They are the sums of the effects from all the elementary monochromatic radiations $E(\lambda)d\lambda$ present in the incident flux. The three receptor systems have here the three functional spectral sensitivities.

The three integrals $\bar{\rho}$, $\bar{\gamma}$, $\bar{\beta}$ give an objective measure of the physico-chemical processes occurring in the retina in response to radiation. These numbers are therefore the best physiologically based co-ordinates for the colour of the radiation. They specify the three signals passed to the brain and, taken together, give rise to the colour sensation.

The new anomaloscope was designed from the concepts expounded above, using, in particular, the $\rho(\lambda)$, $\gamma(\lambda)$, $\beta(\lambda)$ curves obtained by Yustova [1] using a method proposed by Nyuberg [2]. They were fully confirmed in practice on this apparatus. This is significant because our earlier [3] attempt to use Judd's [4] curves showed that the latter were unsound.

(3) Four tests can be performed with the anomaloscope. Three are concerned with colour differentiation, in which the power to distinguish colours ("acuteness of

* Biofizika **2:** 6, 734–742, 1957 [Reprint Order No. BIO 97].

colour differentiation") was measured, as being an important quality primarily relating to the central nervous analyser. The same, in essence, occurs with the various test tables such as Shilling's, Ishihara's or Rabkin's, with the difference that the colour contrasts are given in, so to speak, frozen form.

In our anomaloscope the tests of colour differentiation acuity are done on colour differences which vary continuously and smoothly (rather than by fixed steps), starting from exact colour matches. From the colour differentiation thresholds so based we can derive a precise representation of all colour differentiation acuity variations found.

There must be three such tests because in a normal eye there are three receptor systems to be tested (a statement which must now be accepted as true), each of which participates in the act of differentiation. This is an original feature of our instrument, in which it differs from all others.*

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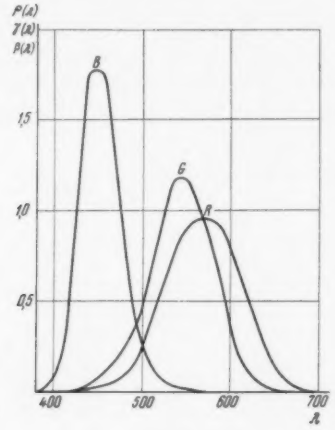

FIG. 1.

(4) In the first test the colour differentiation acuity is estimated for the first receptor system, i.e. that with the spectral curve $\rho(\lambda)$ (Fig. 1), which is the defective one in protanopes. The second and third tests do the same for the systems with $\gamma(\lambda)$ and $\beta(\lambda)$, which are defective in deuteranopes and tritanopes.

The scheme of Fig. 2 explains how our anomaloscope tests each system *separately*, although at first sight this might appear difficult because the spectral curves (Fig. 1) overlap. Fig. 2 represents schematically the effects on the three systems when some colour C_i is observed (left diagram). Here the values of the three integrals above are given for C_1, i.e. the colour co-ordinates ρ_1, γ_1, β_1, in the fundamental physiological system.

It is readily seen that a colour C_2 exists which gives the diagram on the right in Fig. 2. This C_2 differs from C_1 only in ρ, the other co-ordinates being the same; it

* This new instrument differs in principle from that designed and constructed by Demkina [5], assisted by Nyuberg, in 1934. The difference is that in the latter the initial colour match between two half-fields did not correspond to exact identity of spectral composition in all three tests. This is three Rayleigh-type matches superimposed. Here acuteness of colour differentiation only affects the broadness of the match setting, shifts in the match indicating anomalous trichromats.

will differ sharply from the first, e.g. as purple-blue differs from green. The differentiation appears due solely to the difference in signals from the first receptor alone.

On passing from C_1 to C_2 the first system is more stimulated, the stimuli to the others being unaltered. If we now determine what increase in stimulus to the first system ($\Delta\rho$) is at the threshold of perception we get an estimate of the signal difference acuity for the first system alone.

The same can of course be done for the other two systems.* This is clear from Figs. 3 and 4.

(5) The layout of the apparatus is shown schematically in Fig. 5, Fig. 6 being a general exterior view.

The heavy base and column carry the triangular housing, the eye tube projecting from the front. The obtuse angle of the triangle encloses the cylindrical light-source vessel. The white cavity within this vessel contains a 75 W lamp L. The light emerges from the two holes O', O'', and reaches the lens pairs $L'L'$ and $L''L''$, which give bright spots on the screens S', S''. The rays scattered from S' and S'' pass through the prisms P', P'' and through L''' into the eyepiece L_0. This latter enables one to focus the near surfaces of P', P'' and the gap separating them. The field of view is thus composed of a circle with a dark central strip. Between the two L' (left) there is

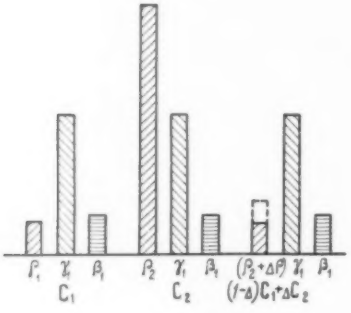

FIG. 2.

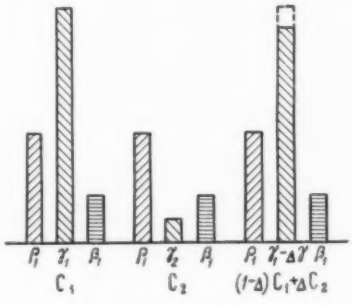

FIG. 3.

a rotating disk D' carrying filters which produce the C_1 for the first three tests in the left field. Two of these filters (F_1 and F_2) are of exactly the same blue-green colour (to remove possible simulation). That of the third is lilac-blue. The filters are changed by rotating D' via the knob B' which shows the test number (or the number of the receptor system under test).

Between the L'' lenses (right) there is also a rotating disk D'' which carries filters precisely the same as D' (F_1, F_2 and F_3). Hence by rotating this with the second knob (B'') exactly the same colour can be produced in the right half-field, i.e. the match between the fields can be set precisely. Also D'' can be shifted across the L''–L'' axis.

In addition to F_1, F_2 and F_3, D'' also carries filters F', F'' and F''' which give colours C_2', C_2'' and C_2''' in the right half-field if D'' is set so that the rays pass entirely through one such filter.

* It is of course clear that the increase can be replaced by a decrease without affecting the result (Figs. 3 and 4).

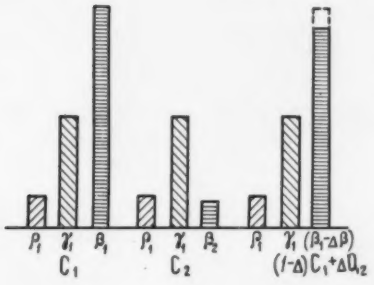

FIG. 4.

As D'' is rotated a smooth change between C_1 and C_2 is seen in the eyepiece. If C_2 is selected as shown in Figs. 2–4 the stimulus to one receptor system only will be affected.

Fig. 7 shows this process in the vector representation. When the ratio $C_1 : C_2$ changes along one of the equistimulus diagonals all colours intermediate between C_1 and C_2 are obtained. The figure also shows that these colours all also lie on the same straight line parallel to the appropriate physiological axis. Hence only one co-ordinate varies in the C_1–C_2 range (e.g. ρ), the others (γ and β) remaining constant.

The third (measuring) knob B_0, which carries a graduated scale, is also used to rotate D''. This gives a reading proportional to the displacement of D'', which is proportional to the shift from the initial C_1 towards C_2, i.e. proportional to the change in the corresponding co-ordinate (say ρ) and hence to the stimulus change to the receptor system.

The first three tests are easily performed on the instrument and are very quick. The apparatus is set to the appropriate marks (1, 2 or 3) on B' and B''. The subject views the field, divided centrally by a black line, which initially has exactly the same

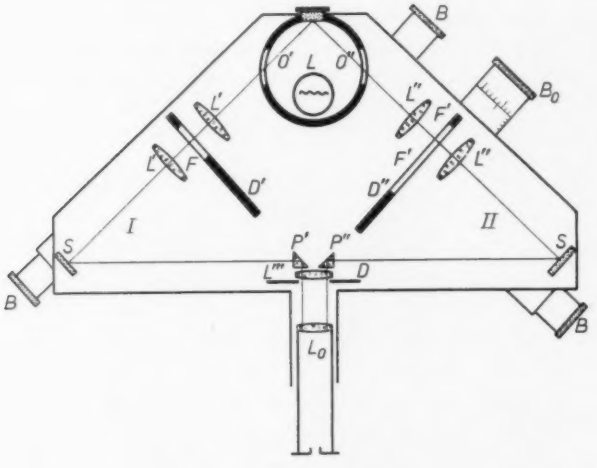

FIG. 5.

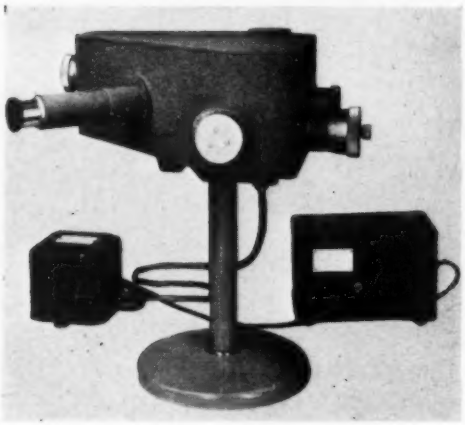

FIG. 6.

colour C_1 in both halves. All the subject has to do is to indicate when an appreciable change in the right half is seen while the experimenter rotates B_0 smoothly to replace F_1 by F'. Only some 3 sec are required for this. The test is repeated 3–5 times to get a reliable mean threshold reading (m). Thus, this device operates specifically as a threshold tester in the first tests.

(6) The fourth test is in principle the same as Rayleigh's [6] which is used in Nagel's anomaloscope [7]. Thus, this test displaces the latter instrument entirely.

For the purposes of the fourth test, D' carries a fourth yellow filter (interference type) with its transmission maximum at 585 mμ. This corresponds to a pair of filters in D'', of red and green glass, selected such that when each covers about half of the aperture between L'' and L'' the colour produced is indistinguishable from the yellow in the left half-field. A special adjustable slide in front of the left yellow filter enables one to adjust the intensity of this beam by rotating B, an exact match in brightness between the half-fields being then also obtained.

The fourth test is thus colorimetric, i.e. involves combining colours to give a colour match. This shows whether the receptor spectral responses are normal or

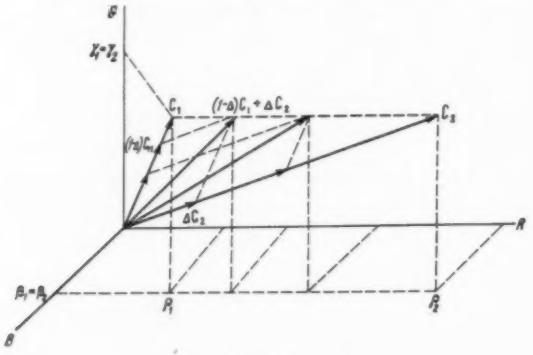

FIG. 7.

show individual deviations in the $\rho(\lambda)$ and $\gamma(\lambda)$ curves, and, if so, in what direction and by how much. It therefore serves to detect and evaluate anomalous forms of colour vision.

(7) The readings a and b obtained in the fourth test fit the equation

$$bC_y = aC_g + (70-a)C_r$$

and hence we get

$$C_g/C_r = a/70-a$$

which, together with b (the reading on B), serves as the fourth index.

If b lies within the normal limits* the value of $a/(70-a)$ should be referred to that found most frequently, $a_0/(70-a_0)$, typical of normals; we thus get an anomaly index, n_A:

$$n_A = \frac{a/(70-a)}{a_0/(70-a_0)};$$

n_A is of course unity for a normal. When $n_A \ll 1$ we have protoanomaly, and when $n_A \gg 1$, deuteranomaly.

The readings from the first three tests, m_1, m_2 and m_3, give a measure of the acuteness of differentiation for the signals from the three receptor systems. To estimate the differentiation acuity aside from any peculiarities of the instrument we may, to a first approximation, refer the readings m_R, m_G, m_B to the appropriate (most frequent) type values. It is, however, more correct to take instead the ratio of the number of thresholds (N) falling in the ranges $m_i(N_i)$ and $m_0(1)$ on the scale, i.e. to define the differentiation acuity indices as

$$n_i = 1/N_i; \ (i=R, G, B).$$

For graduating the anomaloscope scale in thresholds we recently constructed a special device (a double anomaloscope) on which the colour differentiation thresholds could be determined step by step over the whole range from C_1 to C_2, i.e. for all 70 divisions on B_0. This provided $N_i=\psi(m_i)$ curves for all the first three tests (Fig. 8) from which to derive N_i from m_i and hence the differentiations acuity indices for the receptor systems, n_R, n_G, n_B. The thresholds fall outside the scales with complete dichromats. The colour differentiation acuity of the non-functioning receptor system is of course reckoned as zero.

Dichromats can also be tested in the fourth way, by using numerous match settings, when it is found that any mixture of green and red can be matched to yellow by merely altering the amount of yellow (via the slide driven by knob B).

(8) It is clear that this new instrument, and the method developed to go with it, enable one to differentiate types of colour vision by simple tests and to assign values quantitatively. This of itself facilitates a fresh, more precise, classification of colour species.

The number of subjects tested on this instrument has now reached 3000. The data so obtained indicate a wide variety of colour vision types [8], much larger than previously recognized or encompassed by earlier classifications. An extremely

* If this is not so (which is very rare) the anomaloscope readings do not correctly represent the actual colour vision curves because the instrument was not designed for them. Other more complex methods are then required.

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interesting and significant regularity is found: each receptor system can vary within very wide limits independently of the other two. In any given system the differentiation acuity may be 2–3 times greater than normal in one individual, while in another it may be below normal or even zero (dichromat).

Cases are found, by no means uncommon, where two systems are of reduced sensitivity to various extents. Sometimes all three systems are of reduced sensitivity, even by factors 5–6.

(9) As regards cases of deviations from the type curves of spectral sensitivity in the receptor systems, which give anomalous Rayleigh matches, i.e. anomalies, the same may be said [9]. The anomalies, which also vary very widely, do so also independently of variations in differentiation acuity. The two effects are sometimes found together, sometimes in isolation [9]. Cases are common where the thresholds

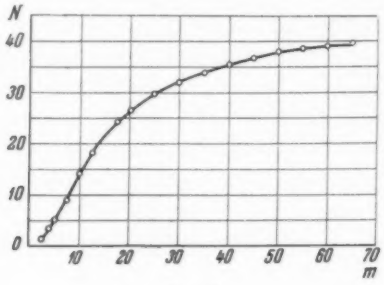

FIG. 8.

are normal or even lower than normal (superacute differentiation) while the fourth test shows a marked anomaly. Conversely, when the thresholds are high, n_A is frequently close to unity, i.e. differentiation is normal. Similar relationships were also noted by Rayleigh [6], who discovered anomalous colour vision in the 1880s.

The term we have proposed for the above effect, i.e. colour weakness, is intended to denote all cases of partially reduced differentiation acuity in any species of receptor [9]. We then get the following gradations:

normals, various degrees of colour weakness in some species of receptor (proto-, deutero-, tritanopia), →
dichromats (colour blind in one receptor), i.e. proto-, deutero-, tritanopia, →
monochromats (completely colour blind in two receptors).

We can also get two types of anomaly (protoanomaly and deuteroanomaly) appearing to various extents. The grouped indices may be split up in the following way:

$(n_R+n_G+n_B)<3$ . . . . . above normal
$3\leqslant(n_R+n_G+n_B)<5$ . . . . . normal
$5\leqslant(n_R+n_G+n_B)$ . . . . . colour weakness.

In parallel with this, the large-scale Rayleigh tests enable us to split up the n_A values as follows:

$0{\cdot}9<n_A<1{\cdot}1$ . . . . . normal
$0{\cdot}8<n_A<0{\cdot}9$ . . . . . tendency to protoanomaly

$1{\cdot}1 < n_A < 1{\cdot}5$ . . . . . tendency to deuteroanomaly
$n_A < 0{\cdot}7$ . . . . . protoanomaly
$n_A < 1{\cdot}5$ . . . . . deuteroanomaly.

In conclusion we present a few cases illustrating the use of the above norms with various types of colour vision revealed by our anomaloscope.

The columns labelled n_R, n_G, n_B give the indices for the R, G, B receptor systems respectively, Σ_n being the sum of these. The next column gives n_A and the last the type of colour vision.

No.	n_R	n_G	n_B	Σ_n	n_A	Colour vision type
1	2	3	4	5	6	7
1	1·3	1·0	0·9	3·2	0·95	Completely normal
2	1·0	1·1	1·1	3·2	2·0	Deuteroanomaly with normal differentiation acuity in R, G and B
3	1·1	1·1	0·9	3·1	0·7	Weak protoanomaly with normal differentiation acuity in R, G and B
4	0·9	1·1	2·5	4·5	4·2	Deuteroanomaly with reduced B acuity
5	3·1	1·3	0·8	5·2	1·0	Colour weakness in R
6	1·1	1·8	5·0	7·9	0·95	Colour weakness in B
7	12·5	0·9	0·8	14·2	1·0	Marked colour weakness in R
8	—	6·5	1·2	—	1·0	Protanope with additional colour weakness in G
9	0·9	—	1·2	—	1·0	Deuteranope
10	4·5	6·0	5·5	16	1·1	Colour weakness in R, G and B

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FREQUENCY CHARACTERISTICS OF THE MASKING EFFECT*

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(*Received* 14 *February* 1957)

MANY experiments have shown that the auditory system performs a frequency analysis of the sound stimulus by using the variation in site of maximum oscillation in the membrane with frequency [1–5], which causes a corresponding variation in the neurone groups excited in the auditory pathways [6–9] and in the stimulus to the terminal region [10–12].

The most important characteristic of the auditory system, considered as a frequency analyser, is its resolving power. The resolving power is usually measured via the frequency characteristics of effects related to spatially restricted regions of the auditory system. Thus, Bekesy [12–13] determined the frequency responses of five points on the membrane by measuring the amplitudes of vibration at different frequencies; Tasaki, Davis and Legouix [14] studied the frequency variation of microphony in various sections of the cochlea; Tasaki and Davis [7] determined the frequency variations of the electrical responses of single nerve cells in the centre of the cochlea using a threshold method [7]; and Tasaki [6] did the same for single nerve fibres. The curves found by Tasaki [6] and Tasaki and Davis [7] are sharper than those of Bekesy [13], indicating heightened resolving power even in the first stages of analysis (a possible mechanism for this is discussed in [15, 16]).

It is of some interest to compare these frequency curves with the final ones determined by "psychophysical" experiments. The end-effect to be measured, known to be related to processes in some limited region of the auditory system, can be the masking effect. Much work indicates that masking is due to the masking sound acting on some limited region of the membrane or on centres concerned with perceiving the masked sound [17–21].

Unfortunately the frequency curves for masking (i.e. those relating the masking of some fixed tone to the masking tone frequency) have not been determined and cannot be computed from Wegel and Lane's data [17] without making additional assumptions. Fletcher's data [18, 22, 23] also cannot be used. Fletcher observed that on widening the masking noise band the effect on a tone at the centre of the band first rose but ceased to rise beyond a certain width. This was shown by the noise having no effect on the masking beyond some critical band-width, and it was further assumed, without a special experimental test, that the tone intensity required for audibility was proportional to the band-width within the critical range, if the spectral level of the noise was constant.

This is clearly equivalent to assuming a rectangular spectral curve for masking. Schafer *et al.* [24] have shown from their studies on the effect of noise band-width on

* Biofizika **2**: 6, 743–755, 1957 [Reprint Order No. BIO 98].

masking that it is more correct to assume a normal resonance-type curve with a frequency-dependent Q. Here again the agreement between theory and experiment was poor for tones lying outside the noise band.

We therefore felt it desirable to study this topic, making the following simple assumption.

It is clear that if a signal is to be heard when noise is present, the stimulus produced by the signal and the noise $y_{n,s}$ to some area Δx of the auditory system should exceed by some amount Δy the stimulus $|y_n|$ produced by the noise alone. If a steady pure tone is used at fixed strength with different noise frequencies (either a pure tone or a narrow noise band) which are increased in intensity till the signal can only just be heard, this latter level will of course correspond to y_n at Δx.

Thus, the noise level giving the same effect at different frequencies can be determined. This can be considered as a threshold method of determining the frequency curve for y_n at Δx. The direct method, on the other hand, consists in keeping the noise intensity constant. Then y_n is determined as the signal intensity which is just audible. It will be shown that the frequency curves obtained by these two methods are somewhat different.

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METHODS

In theory the most exact measurements of the frequency variation of masking are obtained if the signal and interfering sound are pure tones. But the work of Wegel and Lane [17] has shown that the masking effect curves are then complicated by the occurrence of beats when the masking sound is close in frequency to the signal or one of its harmonics. Much more regular curves are obtained by using a noise band as the masking sound [24, 25]. So we used a narrow band of synthetic noise composed of five tones differing one from another by 1–3 c/s and covering a band of 10–15 c/s. The useful signal was a pure tone. Five ZG-2 generators were used in the noise source, these being connected via additional 2kΩ resistors to the attenuator input. A fixed-frequency generator provided the useful signal. The signal was passed to an electronic chopper giving a constant signal duration (0·8 sec) and smooth switching (the time to rise to 80 per cent of the maximum amplitude was 0·03 sec). The output from the chopper was passed through a band filter and attenuator to the same TD-6 earpiece as the noise. The attenuators were worked into their normal output loads by including balancing resistors in the output circuits. The earpiece was calibrated using an "artificial ear". Varshavskii and Glekin [26] have shown that the calibrations obtained with artificial and real ears differ substantially at low frequencies. Appropriate corrections [26, 27] were therefore applied. The unevenness in the corrected frequency characteristic did not exceed ± 5 dB from 100 to 5000 c/s.

One of the ZG-2 generators was used as standard in the work. The signal frequency was determined from this by zero beat and all the other ZG-2 generators set with reference to it. The setting was redone each time the noise frequency was changed. When threshold methods were used the signal threshold was first determined in silence, the noise being then cut in and the signal fed in at 2–3 sec intervals. A warning was given before the signal (lamp lighting). The experiments were started at low noise levels and the intensity increased by 1 dB steps until the signal was no longer perceived. The noise level was then reduced until the signal was again heard.

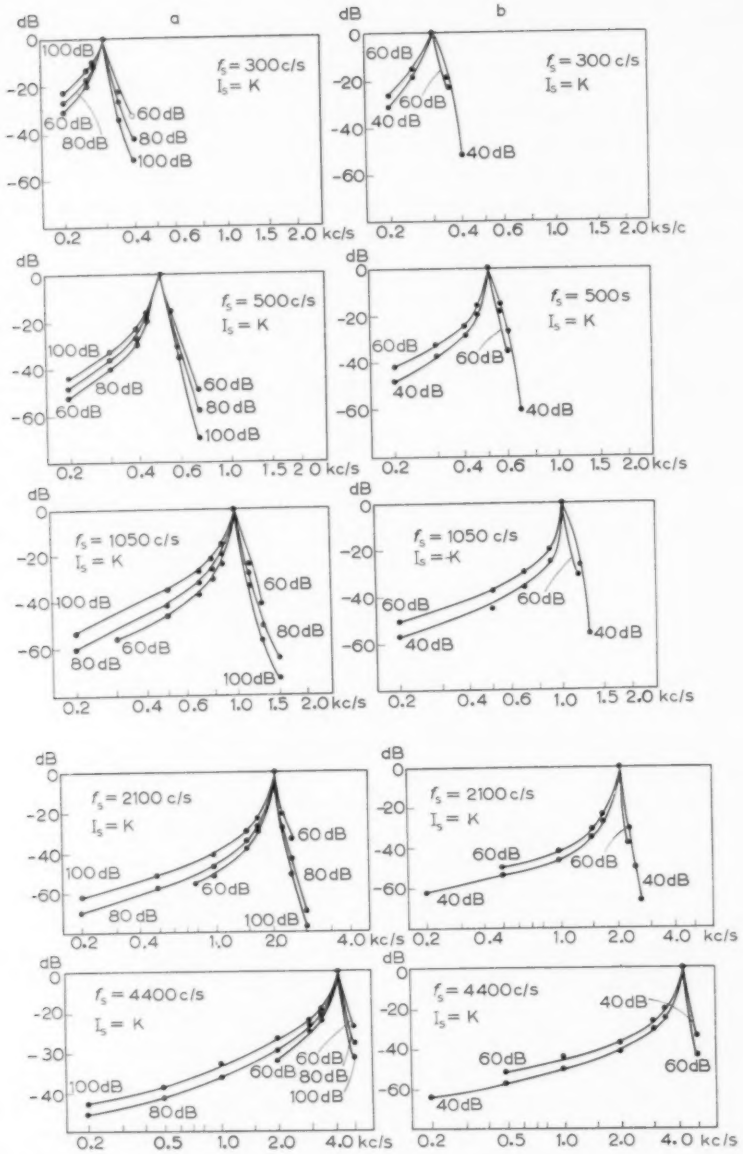

FIG. 1. Frequency curves for the masking effect, as determined (a) by the direct method and (b) by threshold methods.

The measurements were repeated 5–6 times and the noise frequency then changed.

These measurements were done at two signal levels and five frequencies (300, 500, 1050, 2100 and 4400 c/s). When the direct method was used the noise intensity was kept constant and the minimum audible signal level determined. In addition, special experiments in which the noise was composed of four tones separated by 100 or 200 c/s were performed (for details see below). With the narrow-band noise the intensity was taken as being the total power (sum of the components). Seven subjects with normal hearing participated, tests being made on the left and right ears alternately.

RESULTS

It was primarily desired to determine whether the masking effect frequency characteristic remained the same at different noise levels and was the same as those found by direct and threshold methods. Tests at a limited number of noise frequencies sufficed for this. The direct measurements were made at three noise levels (60, 80 and 100 dB), those by threshold at two signal levels (40 and 60 dB). Fig. 1(a) and (b) shows the results. At low noise levels (60 dB) the frequency curves are almost symmetrical, the slope of the right branch increasing and that of the left decreasing on increasing the noise level. This agrees with the observation of Wegel and Lane [17] that the masking at tones above the masking frequency rose more rapidly than that for tones below when the masking sound intensity was increased.

Since the system characteristics depend on the intensity level (i.e. the system is non-linear) it is clear that frequency curves found by direct measurement and by threshold should not coincide. This is clearly visible from Fig. 1. The figure also shows, however, that the differences are very slight at noise frequencies close to the signal frequency. Hence, in refining the curves near the signal frequency, we used threshold measurements as these are more convenient in practice. The signal level was 40 dB.

Preliminary tests showed that at these frequencies the subject can detect the signal in various ways. Thus, when the frequency difference is 50–100 c/s the signal was detected by the buzz produced. At larger frequency differences and with the noise frequency below that of the signal the signal commencement was initially detected as a reduction in noise intensity, and only at high signal levels could the accompanying sound be heard.

With six subjects we determined the differences between noise and signal levels required for the buzzing or fall in noise level to be perceived, and that for the sound to be heard as such. Fig. 2 gives the data. When the signal frequency is 4400 c/s the signal can still be detected as a reduction in noise level at 3800 c/s, i.e. if the frequency difference is 600 c/s. The apparent drop in noise level may be thought to be due to beats which, when of high frequency, determine the perception of the sound level [27–32]. It is interesting that our data [27] give the upper modulation frequency limit at which this effect determines the perception of the intensity of an amplitude-modulated sound (at a carrier frequency of 4000 c/s) as being somewhat greater, between 700 and 1000 c/s.

The next problem was to determine precisely the difference in signal and noise levels when the two frequencies were the same. In fact we found much accentuated masking in these cases. The subject heard the signal only when it was of the same

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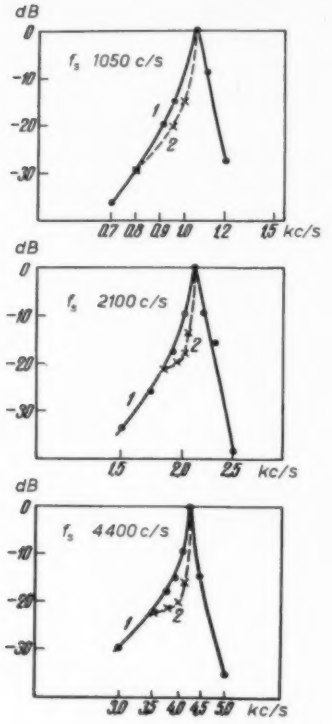

FIG. 2. Frequency curves for the masking effect at frequencies close to that of the signal. (1) difference in signal and noise levels at which the signal is heard as such (high sound appears); (2) difference in signal and noise levels at which the signal is heard by virtue of the drop in noise intensity.

intensity as the noise, i.e. when the total power was twice the noise power. This determination is merely one of the intensity differential threshold [33].

Several papers [34–38] show that the differential threshold for pure tones and noise at medium intensity levels is about 0·3–0·7 dB, which corresponds to a power increase of 1·072–1·175 times, i.e. much less than found in our experiments.

To understand this discrepancy we must remember that the noise we used was composed of five tones of equal intensity lying in a band 10–15 cycles wide. This noise is heard as a pulsating tone (beat frequency about 10–15 c/s) with an irregularly varying intensity. It is therefore more difficult to detect the signal because not only must the intensity increase (due to adding the signal) be detected, but it must also be differentiated from the random possible noise variations. This must naturally result in the masking being accentuated.

Additional experiments are required to determine the true relations between the masking effects occurring when the signal and noise frequencies are the same and different; in these the same criterion must be used in both cases. An experiment which approaches this is to compare the timbres of two complex sounds. If a complex sound, consisting of several tonal components, has added to it either a fresh

component or merely more of one of the existing components the new signal can only be differentiated from the original by the change in timbre. Such an experiment differs from ones on masking in that here the component parts of a complex sound, which in the case of masking are termed signal and noise, are present simultaneously, and cannot therefore be separated [39]. Results on masking in these conditions, when compared with those from normal masking, show that the absolute values differ but the frequency variations are identical [40].

To study the masking of a 1050 c/s tone by noise of the same frequency we used a five-tone sound, containing 850, 950, 1050, 1150 and 1250 c/s with intensity levels of 68 dB. Fig. 1 shows that the differences of 100 c/s are sufficient to ensure that the masking of the 1050 c/s is due solely to the component with this frequency. This complex sound is compared with another identical in all but the level of the 1050 component (which was increased). From six subjects the mean minimum increase in the 1050 component intensity at which the sound was differentiated in 75 per cent of the cases was 4·0 dB, corresponding to a 1050 component power increase of a factor 2·512. If we consider the power increase as our wanted signal the corresponding difference in the signal and noise is +1·8 dB.

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To measure the masking at noise frequencies differing from the signal frequency we used sounds of the following compositions: 1200, 1300, 1400 and 1500 c/s; 600, 700, 800 and 900 c/s; 500, 600, 700 and 800 c/s; and 200, 400 and 600 c/s. Without much error we can take it that the masking of the 1050 signal is entirely determined by the closest component. By alternating the presentation of these sounds and the sounds + signal (1050 c/s) the minimum 1050 tone levels at which the sounds were differentiable were determined.

The mean differences (for six subjects) in the levels of signal and noise found are shown in Fig. 3 (curve 1). Comparing 1 and 2 (masking by synthetic noise at a level of 80 dB) we see a rise in the masking in the timbre determination experiments of about 9·0 dB on average.

Check experiments in which the same compound sounds were used as in the timbre experiments, but with the 1050 component switched on later than the others, showed that the masking dropped by about the same amount (9·3 dB, mean of 10 measurements). The differences between curves 1 and 2 are thus caused by the differences in the switching used.

Assuming a correction of 9 dB to allow for the difference in conditions, we could then determine the true masking on 2 (using curve 1) when the noise and signal were of the same frequency. This was $+1{\cdot}8-9{\cdot}0 = -7{\cdot}2$ dB. This corresponds to an increase in sound power (on adding the signal) by a factor 1·190 or a rise in intensity level of 0·8 dB. The differential intensity threshold for a trained observer is somewhat less than this. Since these differential thresholds are about the same over the frequency range we used we can apply the same correction to the other four curves. These corrected curves, determined by the direct method at 60 and 80 dB noise levels, are shown in Fig. 4. The curves are the means for seven subjects (14 ears). A study of the deviations from the mean shows that the figures for the individual subjects always show a much narrower scatter when the noise frequency is below the signal one. The mean σ_m when $f_n < f_s$ is 0·8 dB, the error of the mean being 2·2 dB when $f_n > f_s$.

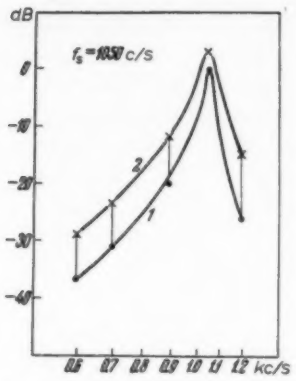

FIG. 3. Frequency curves for the masking effect determined by different methods. Explanation in text.

It is of some practical interest to see whether these frequency curves can be used for calculating the masking of the components of a compound discrete-spectrum sound. For calculation purposes we can, as others have done [24, 41], assume the auditory system to be an assembly of filters with frequency characteristics corresponding to those of the masking effect. Further, we can from our data assume that in order to detect a signal we must have a difference in noise and signal levels at the output of a filter tuned to the signal of +1·8 dB. Then from Fig. 4 we can calculate at what levels components of frequencies 300, 500, 1050, 2100 and 4400 c/s can be detected in sounds of known spectral composition.

The calculated and experimental data are given in Table 1 (the experiments consisted in comparing timbres). It is seen that the agreement between theory and experiment is satisfactory when the wanted component falls at medium and high frequencies. If the wanted frequency falls at 300 or 500 c/s the two only lie close together if the wanted one is of a frequency higher than the other components. The largest discrepancies occur when the wanted component lies in the centre of the distribution. A 300 cycles tone is so much more masked by a combination of 100, 200, 400 and 500 cycles tones than by ones containing 100 and 200 or 400 and 500

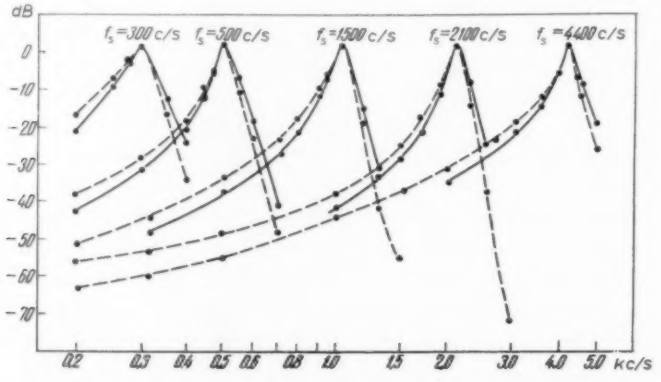

FIG. 4. Frequency curves for the masking effect, corrected.

TABLE 1. COMPARISON OF EXPERIMENTAL AND CALCULATED RESULTS

Signal frequency (c/s)	Frequencies of components (c/s)	Levels of noise components at the input (dB)	Levels of noise components at the output (dB)	Level of total noise power at the output (dB)	Signal threshold	
					Calculated (dB)	Experimental (dB)
300	100	70			50·8	53
	200	70	49	49		56 mean 56
						57
						57
	400	72	43	43	44·8	53
	500	74				53
						51 mean 54
						56
500	100	60			41·4	61
	200	60	37	39·6		57
	400	62	36			47 mean 55·5
	500	64				57
	100	70			52·2	56
	200	70	28	50·4		57 mean 54
	300	71	39			44
	400	72	50			62
1050	300	61	28		47·3	59
	400	62	40	45·5		54 mean 56
	600	65	44			53
	700	67	24			59
2100	850	60	49		63·8	55
	950	69	61	62		60 mean 64
	1150	68	53			69
	1250	67	35			71
4400	1300	66	30	51·6	53·4	43 mean 54
	1500	65	35			54
	1700	64	41			61
	1900	63	51			57
	3600	65	48	61·6	63·4	57
	3800	63	52			66 mean 63
	4000	64	56			59
	4200	62	50			69

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that in general we cannot conceive of a filter capable of giving such results. It is clear that our model is inadequate.

DISCUSSION

It is important to compare our data with those of others. This can only be done for the results of Wegel and Lane [17] at signal frequencies of 1000 and 2000 c/s, which lie close to ones we used. From the graphs given by Wegel and Lane for masking tones of various frequencies, we used those showing the relation of the masking of 1000 and 2000 c/s tones to the level of masking sound sensation. These data were

transferred to absolute intensity levels, for which we used Sivian and White's threshold curve [42]. We then determined the levels of 1000 and 2000 cycles tones at which they masked various tones at the levels of interest to us (60 and 80 dB). To derive the signal level when the noise and signal were of the same frequency, which Wegel and

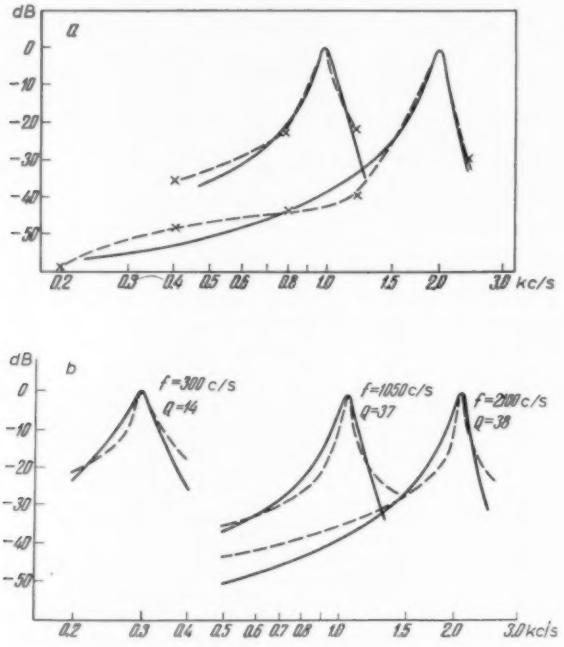

FIG. 5. Frequency curves for the masking effect taken from the data of various workers.

Lane did not do, we assumed, in accordance with our data, that the signal is here masked when its level was 7 dB below that of the noise. Fig. 5(a) shows the frequency curves for masking so obtained (the points taken from Wegel and Lane being shown by crosses). The curves are obviously very similar.

For comparison with the curves of Schafer *et al.* [24] we have drawn up in Fig. 5(b) resonance curves corresponding to the Q-values found by these workers, who determined Q at three signal frequencies: 200 ($Q=9{\cdot}3$), 800 ($Q=37$) and 3000 ($Q=39$). In Fig. 5 we have assumed $Q=14$ at 300 c/s, which corresponds to Q increasing linearly from 200 to 800 c/s. Our experimental curves obviously deviate very considerably from true resonance shape. It is also clear that to obtain better agreement between the resonance curves and our data at frequencies close to that of the signal it is necessary to use lower Q's. It is true, of course, that this still further increases the discrepancies at the wings of the curves. It is of some interest that Pumphrey and Gold [43] found Q values much larger than Schafer *et al.* from the time response of auditory perception (50 at 1000 cycles and 200–350 at 2500–10,000 cycles). This is clearly quite at variance with our data and is evidently due to the time characteristics

originating mainly from processes in the nerve links of the auditory system and not directly reflecting the response of the auditory organ as a frequency analyser.

Bekesy's [13] and Tasaki's [6] frequency curves are shown in Fig. 6 for comparison with our data. For convenience Bekesy's curves ([13], p. 250) are shown on a log scale, and Tasaki's threshold curve is turned round. Our curve, shown in Fig. 6, was taken by threshold methods at 60 dB. It differs greatly from Bekesy's; its shape has a similarity to Tasaki's. Ours differs from Tasaki's in showing a much steeper fall on the low-frequency side. Hence low frequencies are much less effective in masking than would be expected from the response of a single nerve fibre.

It has often been assumed that the nerve links increase the resolving powers of the analysers [44]. But the frequency responses of the single nerve cells of the cochlea are no sharper [7]. Hence, if the resolving power is in fact increased in the nervous system, the increase must take place at higher levels.

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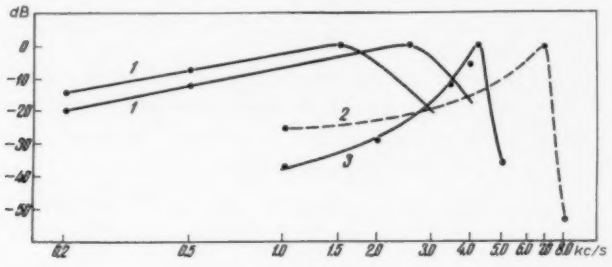

FIG. 6. Frequency curves for the masking effect in the auditory system. (1) vibration amplitude of the basilar membrane (after Bekesy); (2) response of a single nerve fibre (Tasaki); (3) masking effect.

An interesting aspect of the frequency variation of masking is, as we noted above, that it depends on the intensity; as the noise intensity increases the curves become less symmetrical. The rise in the masking effectiveness of low tones with intensity is usually [17, 23] explained by additional masking produced by the harmonics resulting from nonlinearities. Distortion of noise of frequency lower than the signal may of course in principle cause additional masking (because harmonics closer in frequency to the signal are produced from the noise), while distortion of noise of higher frequency than the signal should have no effect. It must of course be assumed here that the distortion occurs at some stage or stages prior to the analyser because the noise spectrum reaching the analyser is already complex.

However, it is difficult to make these explanations agree with the experimental results. It is quite clear that noise of frequency 1500 c/s becomes more effective in masking a 2100 c/s tone as its intensity rises. The above hypothesis would assign this to additional masking by noise harmonics. But the first harmonic (3000 c/s) already lies above the signal, and can, from Fig. 1, produce no appreciable extra masking.

It is more probable that the observed relation of masking curve shape to noise level is due to nonlinearities in the analyser itself (cochlea system). Thus Tasaki, Davis and Legouix [14] found from the microphonic effect in various parts of the

cochlea that in any particular section the relation of the amplitudes of the potentials to the stimulus amplitude was frequency-dependent. At low frequencies the relation was linear over wide ranges, but became nonlinear at high frequencies, the linear region growing shorter with rise in frequency. The more remote the area of the cochlea, the lower was the upper limit to the frequency range in which the amplitude response was linear.

The frequency curves for this effect found at different stimulus levels therefore differ in shape. The higher the intensity, the less effective are the high frequencies relative to the low ones.

The data of Davis *et al.* not only indicate that the amplitude-dependence of the masking curves is occasioned by nonlinearity in the cochlea elements, but also contra-indicate any explanation in terms of conducting element nonlinearities. Since non-linearities were found only at very high levels at low frequencies it is clear that the conducting system is linear over a very wide intensity range.

SUMMARY

(1) The frequency variation of the masking effect has been studied at five signal frequencies (300, 500, 1050, 2100 and 4400 c/s). Narrow-band synthetic noise (five tones lying in a 10–15 c/s band) was used. The signal frequency was kept constant and the noise frequency varied in the experiments.

(2) The results show that these frequency curves differ from simple resonance form.

(3) The shapes of the curves depend on the intensity level. At low noise levels the curves show a sharp drop on the high frequency side and a slow drop on the low frequency side.

(4) The sharpness of the curves near the signal frequency increases with the signal frequency itself.

(5) If the curves are used to calculate the masking of the separate components of a discrete-spectrum sound the results are in satisfactory agreement with experiment provided that the component in question lies at medium or high frequencies.

Translated by J. E. S. BRADLEY

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DISCUSSION

ON THE NATURE OF THE ELEMENTARY PHOTOREACTIONS IN CHLOROPHYLL*†

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(*Received* 8 *January* 1957)

IN Krasnovskii's paper [1], which is to a certain extent a review of a great deal of work published in 1946–1948 [2–4], the essentially new concepts on the biradical, long-lived, molecular triplet state [5–8] are widely used. These concepts, which in

† Reported at the All-Union Conference on Chemical Kinetics and Reactivity, held in Moscow in June 1955.

* Biofizika **2:** 6, 756–763, 1957 [Reprint Order No. BIO 99].

the papers are related to the problem of photosynthesis, are extended to the oxidation-reduction reaction of chlorophyll in the process of its sensitizing action. At the present time, however, experimental material has collected which shows a wider range and variety of properties of chlorophyll and therefore, in our opinion, some important corrections should be put forward to the ideas developed in those papers.

Thus, applying the hypothesis of the biradical long-lived molecular state to the photoreaction of chlorophyll, Krasnovskii, when discussing photoreduction, writes, in conformity with these ideas:

$$\cdot X\cdot + AH \rightleftharpoons X^- + \cdot AH^+ \qquad (1)$$

or, for photo-oxidation reactions:

$$\cdot X\cdot + K \rightleftharpoons \cdot X^+ + \cdot K^- \qquad (2)$$

where X is a chlorophyll molecule with its magnesium atom exposed, i.e. no "addendum" attached; $\cdot X\cdot$ is the same chlorophyll molecule but in the "bivalent", and according to Terenin, photoactive state; AH is a reducing agent, K an oxidizing agent.

It is supposed that the excited molecule, having received a light quantum, goes over to the biradical long-lived state by reversal of the spin of a π-electron in the system of conjugated bonds of the porphyrin nucleus of the molecule according to the reaction:

$$X + h\nu \rightarrow X^* \rightarrow \cdot X\cdot.$$

The two valencies, thus arising, then react in suitable conditions with a reducing or oxidizing agent according to schemes (1) and (2).

The sole* experimental basis of the applicability to this case of the hypothesis of biradical states is formed by experimental data which do not establish a direct connexion between the extinguishing action of certain molecules and their capacity to react photochemically with chlorophyll. For example, quinone does not react photochemically with chlorophyll but it strongly extinguishes the fluorescence of the latter in alcoholic solution; on the other hand ascorbic acid reacts rapidly with chlorophyll in pyridine solution, but nevertheless has no influence on its intensity of fluorescence. These facts, in the author's opinion, indicate a reactivity not of an electronic excited state having a life of 10^{-7}–10^{-8} sec, but of a long-lived biradical (triplet) state [9].

There would be no doubt about all this if the molecules of these pigments were capable of interacting (photoreacting) with other molecules in one way only, namely by means of the system of conjugated bonds of the tetrapyrrolic nucleus of the molecules of these pigments, as Krasnovskii and his co-workers consider. But the experimental fact, shown in the same study [9], of the complete correspondence between extinction of chlorophyll fluorescence by oxygen and photoreaction with it, would not be understood at all. Moreover, this experimental fact in itself shows that the chlorophyll molecules participate in the photoreaction not in a metastable (triplet) state, but in an excited state.

It therefore follows that the experimental data of this work [9] cannot serve as sufficient argument, either for or against, the ideas developed in paper [1]. In order to form correct conclusions, deeper investigations are necessary to clarify the mechanisms of extinction and photoreaction of chlorophyll and magnesium phthalocyanine.

* We cannot pay attention to the absorption band at 740–760 mμ which is mentioned by the author of the paper [1], for the result of the measurement has not been verified and has not been published anywhere.

The experimental facts which contradict the concepts developed in reference [1] are not confined to those above. For example, the bleaching on illumination of the system pyridine-chlorophyll-ascorbic acid in anaerobic conditions (and other similar reactions), with the formation in the spectrum of a maximum at 525 mμ (in the region 500–550 mμ), is interpreted by Terenin [10–12], Krasnovskii, Evstigneev *et al.* as indicating the formation of a reduced form of chlorophyll by hydrogenation of the long-lived, metastable, biradical chlorophyll molecule on the system of the conjugated bonds of the nucleus. But well-known experimental facts* show that, when a chlorophyll molecule is thus hydrogenated or protons are attached to the nitrogens of the tetrazoporphyrin nucleus of magnesium phthalocyanine, a sharp displacement of the spectrum takes place from the visible region to the near infra-red, but not to the short-wave region. We see from these facts that the spectrum shift towards long wavelengths takes place for various reactions, both thermal and those activated by light and ultrasound. It is incomprehensible that, with a photoreaction going, as proposed by Krasnovskii, through a metastable biradical and ending with the formation of the reduced form of chlorophyll (i.e. with hydrogenation of its molecule along the conjugated bond system), the spectral effect would differ from the spectral effect observed on hydrogenating chlorophyll in the usual reaction on the same conjugated bond system.

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Hence it follows that the so-called "reduced" form of chlorophyll is indeed either not its reduced form or, if it is, then not on the conjugated bond system of the nucleus of the molecule and consequently not through a metastable biradical.

We think that all such contradictions are a consequence of not paying attention, up to the present, to the concept of chlorophyll (magnesium phthalocyanine) as a complex-forming compound and, in the very important questions of photosynthesis, a molecule of this pigment is considered to be plane molecule with its magnesium atom exposed and lacking addenda.

Chlorophyll and magnesium phthalocyanine are characteristic chelating agents with a magnesium atom in the centre of the molecule. Under normal conditions, and in conformity with the two free co-ordinate bonds of these molecules, the magnesium atom is always connected to two symmetrically situated addenda which must not be disregarded [19–21].

For this reason the writing of a reversible reaction in the form (1) cannot be correct and, when taking over the biradical long-lived molecular state hypothesis for the photoreaction of chlorophyll, we must bear in mind that, in reality, we do not have a molecule of chlorophyll or magnesium phthalocyanine —X— which has an exposed magnesium atom, but a molecular compound $—A_1XA_2$, where A_1 and A_2 are possible addenda for the given pigments† under given conditions.‡

* We have in mind the references [13–17] and also the firmly established position that, when the conjugated bond system of porphyrin molecules is hydrogenated, the spectrum of the latter is displaced towards the long-wave region (approximately from 600 to 700 mμ) and, when hydrogenation takes place on the conjugated bond system of chlorin molecules, their spectrum is displaced still further towards the long-wave region (approximately from 700 to 800 mμ) [18].

† For convenience in exposition, magnesium phthalocyanine is also called a "pigment".

‡ This situation, naturally, refers not only to the work of Terenin, and Krasnovskii *et al.*, but also to the work of other authors who have not paid attention to these properties of the molecules of chlorophyll and its derivatives.

The necessity of considering this circumstance is dictated by a whole series of experimental facts which enable one to state that the molecules of such substances as chlorophyll or magnesium phthalocyanine can interact with their surrounding media in three different ways: (1) by means of the peripheral part of their molecule, (2) by direct interaction with the cyclic system of conjugated bonds of the tetrapyrrolic nucleus of the pigment molecule, and (3) by way of the addenda which are joined, through the magnesium, to these pigment molecules. In all three cases the spectral effects of the reactions will be different.

Actually, from the studies [22–24 and others], those changes in the spectra of chlorins and porphyrins, which take place as a result of substitution of the side groups of the molecules of these substances by other groups, are very well known. With respect to reactions in which the conjugated bond system is directly affected, there is also sufficient material; in particular it is well known, and we have already discussed it above, that their hydrogenation leads to a sharp shift of the spectrum from the visible region to the near infra-red. Finally, our studies [21, 25, 26] give a clear picture of the nature of the spectral changes on substitution of the addenda by others; they show that changes take place in the position of the red maximum in the absorption and fluorescence spectra by amounts up to approximately 40 mμ, accompanied by a rebuilding of the fine structure of the spectrum, and that the absorption and fluorescence spectra, in the visible, of these pigments disappear simultaneously with removal of the addenda, i.e. the pigment is bleached.

From what has been said it follows that, when a pigment molecule absorbs a light quantum, the change in its electronic state can be localized not only in the conjugated bond system but also in other parts of the molecule, and in particular in the system of bonds magnesium–addenda.*

From this point of view the "contradiction" of the experimental data in [9], which we have already pointed out, is inevitable and the explanation of the photoreaction of chlorophyll on the basis of a long-lived, biradical, molecular state [1] cannot be considered as sufficiently conclusive.

The ability of the molecules of chlorophyll and magensium phthalocyanine to react in three different ways, each of which leads to characteristic spectral changes, suggests the possibility of selectively affecting the pigment molecule. By varying the agent acting, one can thus hope to study the reaction mechanism in each individual case.

From this point of view it is of interest to analyse the already existing experimental material and, in particular, our data obtained on adsorbates of magnesium phthalocyanine and chlorophyll; these, in the author's opinion, give the key to understanding the mechanism of one of the above-indicated three cases, namely the case of the interaction of pigment molecules through their addenda and, to a certain extent, disclose the mechanism of fluorescence extinction.

Thus, it has been shown that the intensity of the fluorescence obtained from a solution of an adsorbate of magnesium phthalocyanine (on MgO), measured at 20°, falls with increase in the temperature at which it is liberated in a vacuum from

* In the limiting case this can lead, on absorption of the corresponding quantum magnitude, to dissociation of these bonds and then to the formation of bonds with new addenda. This interesting question demands special consideration.

other molecules, and at a temperature of 375° it completely vanishes [25]. At the same time, as the intensity decreases a gradual splitting of the principal fluorescence maximum into components takes place.

As subsequent experiments have shown, the absorption spectrum of the adsorbate of magnesium phthalocyanine undergoes exactly the same kind of conversion, and it also vanishes after liberation from the other molecules at 375°. The change in the fluorescence spectrum takes place corresponding with the change in the absorption spectrum [21]. Such phenomena, corresponding to its molecular features, also take place with chlorophyll as is seen in the same paper [21].

From these experiments the structure of the principal maxima of absorption and fluorescence of chlorophyll and magnesium phthalocyanine is clearly complex and some components, being destroyed, vanish earlier than others which are more stable. Thus, the fall in intensity of fluorescence [25] is satisfactorily explained by the thermal destruction of various complexes; in parallel with their decomposition, the absorption and fluorescence spectra belonging to them vanish.

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The complex structure of the principal absorption and fluorescence maxima can also be seen from other experiments, namely from experiments on the extinction of fluorescence [27] in which a distinct reaction of these complexes is developed with respect to oxygen; and this can only take place because of the distinct chemical individuality which is conferred by the different addenda. In these experiments it was shown that for adsorbates of chlorophyll and magnesium phthalocyanine (obtained from solutions) previously pumped out in a high vacuum, extinction by oxygen begins only at a partial pressure of oxygen of 60 mm and rapidly increases with further increase in pressure [27].

If evacuation is carried out at gradually increasing temperatures it is found that the higher the temperature of evacuation the greater is the magnitude (on letting in oxygen after cooling the adsorbate to room temperature) attained by the other, opposite, effect—"enhancement" of fluorescence; while the extinction gets smaller and smaller and finally, at a temperature of 375°, completely vanishes, giving way entirely to enhancement.

It is important to stress that enhancement of fluorescence always begins at a completely different oxygen pressure from extinction, namely at very small pressures when the appearance of the spectrum can be detected only by the application of a photometric method. It is also important to note that this happens not only with oxygen but also with all other substances capable of evoking absorption and fluorescence spectra in a pigment by complex-formation with it through the magnesium atom [21, 26]. In all these cases enhancement begins at very low pressures. This circumstance points to the primary nature of the effect of enhancement of fluorescence and the secondary nature of the effect of its extinction by oxygen. Therefore enhancement can in no way be considered a kind of activation of fluorescence, i.e. an effect equivalent, but opposite to, extinction. These processes are quite different in their nature. In the first case we have the formation of an absorbing and fluorescing complex; in the second case, the influence on it of another molecule.

As experiment shows, extinction is observed when oxygen acts on the complex of the pigment with hydrogen-containing molecules such as C_2H_5OH and $(C_2H_5)_2O$, and is never observed when it acts on oxygenous complexes, even at an oxygen

pressure of 1·5 atm. These circumstances explain the gradual increase of enhancement and the gradual vanishing of extinction in our experiments. In fact, on the one hand, with a gradually increasing temperature of liberation, *in vacuo*, of the adsorbate from other molecules, with subsequent cooling to room temperature and the letting in of oxygen, a smaller and smaller quantity of the complexes with addenda of solvent molecules, i.e. hydrogen-containing and capable of undergoing extinction, remains in the adsorbate; on the other hand, the adsorbate becomes more and more rich in complexes with oxygen-molecule addenda which do not undergo extinction of fluorescence. This can be well seen from the experiment illustrated in Fig. 1.

The interaction of these two opposing factors also causes the experimentally observed change in the photometric curves which are gradually converted from

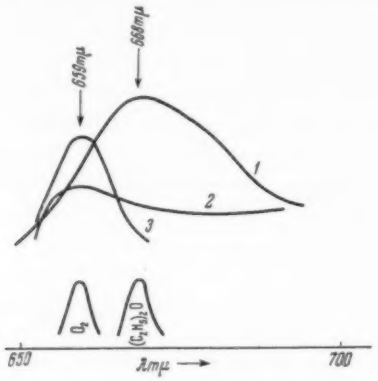

FIG. 1. Change in the spectrum of the principal fluorescence maximum of the adsorbate of magnesium phthalocyanine after successively evacuating with heating, then cooling and letting in oxygen. (1)—original adsorbate, obtained from a diethyl-ether solution; (2)—the same adsorbate after pumping out in a high vacuum at a temperature of 300°, cooling to room temperature and letting in oxygen (P = 20 mm); (3)—the same adsorbate after pumping out in a high vacuum at a temperature of 375°, cooling to room temperature and letting in oxygen (P = 60 mm). Below, for comparison, are given the spectra of the principal fluorescence maxima of complexes of magnesium phthalocyanine with oxygen (650 mμ) and with diethyl-ether (668 mμ) in the adsorbed state.

curves of extinction to curves of enhancement [27]. These experiments also show that fluorescences of one and the same molecule—magnesium phthalocyanine or chlorophyll—can be associated with various chemical properties, according to the wish of the experimenter.

In this way, experiments on the oxygen extinction of the fluorescence of the original adsorbates of chlorophyll and magnesium phthalocyanine, obtained from solutions and heated in a vacuum to various temperatures, discloses the existence in them of fluorescing complexes of distinct chemical individuality. These experiments show the development, both in chlorophyll and magnesium phthalocyanine, of different fluorescences, belonging to molecules of the same pigment and of qualitatively distinct properties. This indicates the integral character of the fluorescence spectrum, and, consequently, also the absorption spectrum,* of each of these pig-

* The interdependence of the absorption and fluorescence spectra in these pigments was shown by us earlier [21].

ments and the varied nature of the spectra of the components, which is due to the different properties of the various addenda forming complexes with the pigment. This circumstance suggests that the spectral properties of a given pigment in the visible region chiefly depend on the addenda, and not on the conjugated bond system of the tetrapyrrolic ring of its molecule. They are as diverse as are the addenda which are capable of forming complexes with the given pigment.

The selectivity of the fluorescence extinction in adsorbates of chlorophyll and magnesium phthalocyanine only with respect to certain addenda, reveals the mechanism of the extinguishing action of oxygen. This selectivity (and not uniformity) in the action of oxygen shows that, when oxygen extinguishes fluorescence, it interacts with the addenda of the complex, and not with the π-electrons of the conjugated bond system.

The mechanism of this phenomenon is subject to further, more detailed, study.

Our experiments, described here, also have another significance: they once more, and by another method, show the existence of fine structure in the principal absorption and fluorescence maxima in the spectra of chlorophyll and magnesium phthalocyanine which is due to complexes of different kinds. If we could succeed, for example, in getting pure alcohol or ether complexes and, in conformity with this, their absorption and fluorescence spectra, which do not possess fine structure, then the latter would immediately begin to develop during the process of chemical reaction of the addenda of these complexes with molecules of air and water vapour [28]. This process and the fine structure of the spectra would exist until the chemical reaction is finished and its product is formed—a stable new complex of the pigment with the addenda which have reacted and, as a consequence, have been transformed. This new complex will now possess other, new, chemical and physical properties.

Thus, from the experimental data on fine structure*, we see that the spectral and other properties of these pigments are due, to a great extent, to the addenda and we must therefore take them into account in a correct theory.

The concepts of a "physical path" and a "chemical path" of photosynthesis, put forward in paper [1], rest, for their theoretical interpretation, on the photoactivation of chlorophyll molecules by means of the formation of a long-lived biradical on the conjugated bond system of their porphyrin nuclei and do not take into account the important properties which this pigment possesses as a complex-forming compound. This account shows that these concepts do not possess sufficient experimental bases, are in contradiction with experiments on extinction of fluorescence and on a deeper theoretical analysis lead to contradictory, diametrically opposed, conclusions.

Interesting experimental data on the change in potential when heterogeneous and homogeneous, chlorophyll containing, systems are illuminated by visible or red light [29, 30], are considered by the author of paper [1] as experimental support for the interpretation of the so-called "photoreduction" reaction of chlorophyll. For two reasons, however, these experimental facts cannot be considered as support for these ideas: (1) results of experiments on potentials cannot be interpreted uniquely at the present time; they can be explained from another point of view with no less success†;

* The fine structure was demonstrated by three different methods: (1) by the dynamics of the changes in the principal maximum while it was being destroyed, (2) by extinction of fluorescence, and (3) by the method described by the author earlier [21].

† The author will discuss this question in a subsequent publication.

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(2) all we have said in this paper also applies equally to the interpretation of the results given in communications [29, 30].

SUMMARY

Recently developed concepts on the photosensitized reduction and oxidation of chlorophyll [1] are discussed in the light of experimental material [9, 13–28]. It is noted that the concepts of "physical" and "chemical" pathways in photosynthesis, which are theoretically based on the photoactivation of chlorophyll molecules by means of the formation of a long-lived, metastable, biradical on the conjugated bond system of their porphyrin rings, do not have sufficient experimental foundations, are in contradiction with experiments on fluorescence extinction and, when more deeply analysed, lead to contradictory conclusions.

The major role played by additions to the magnesium atom in the molecules of chlorophyll and magnesium phthalocyanine, revealed in the studied experimental material, proves that it is necessary to take into consideration the "complexing" mechanism of interactions with other molecules (e.g. oxygen), namely the reactions with the addenda.

It is shown that the maxima in the spectra of chlorophyll and magnesium phthalocyanine in the visible region possess a structure whose complexity is determined by the various possible addenda, and that the extinction of pigment fluorescence by oxygen is dependent on the interaction of the oxygen with hydrogen-containing addenda and not with the conjugated bond system of the molecules of chlorophyll and magnesium phthalocyanihe, i.e. not with the unsaturated valencies of the metastable biradical.

Translated by J. DAINTY

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LETTER TO THE EDITORS*

REGARDING GRAEVSKII AND KEILINA'S PAPER "REDUCED SENSITIVITY TO LETHAL DOSES OF X-RAYS IN ANIMALS PREVIOUSLY GIVEN SUBLETHAL DOSES"†

THE present importance of the rise in the resistance of living organisms to ionizing radiations is not in doubt. In this respect the paper is of interest. Considerable increases in the resistance to X-rays were obtained on first giving white rats and dogs nonlethal doses. They consider 500 r of filtered X-rays given as a single whole-body dose sublethal in dogs and white rats. All survived at these first doses. After 150 days, lethal doses, taken as being 1000 r, were given. At this second dose 55 per cent of the rats survived to day 36, all the dogs surviving, while the control groups given one dose of 1000 r all died. Unfortunately the numbers of controls and their times of death are not given for the dogs, although Fig. 1 indicates that some dogs were alive after 70 days.

We do not object on principle to there being a rise in resistance to penetrating radiations following prior irradiation. The effect is well known. We have also found it. But some points in the paper give rise to serious doubts.

(1) We cannot agree that single-shot whole-body 500 r doses are sublethal in rats (even less so in dogs), or that then only brief radiation sickness is produced in dogs, as claimed on p. 235: "the ones given 500 r remained active, ate their food well and did not lose weight".

Published data (Gorizontov, Shapiro, Nuzhdin, Tarusov, G. A. Brecher, E. P. Cronkite, W. A. Clarke, R. Uncapher, T. C. Evans), the "Official instructions on methods of X- and γ-raying animals, U.S.S.R. Ministry of Health, 1954", and our own studies, have shown that LD 50/30 for rats varies from 450–640 r with a minimum lethal dose of 250–300 r, the values for dogs being 300–400 r and 275 r.

(2) There is no doubt that 1000 r falls beyond the absolute lethal limit for rats, and more so for dogs. Gorizontov's data indicate that 600 r approaches the absolute lethal level for dogs (mortality 96 per cent).

In our experiments on antibiotic treatment, of 13 dogs (500 r doses given) 5 died

† Biofizika **1:** No. 3, 1956.

* Biofizika **2:** 6, 764–765, 1957 [Reprint Order No. BIO 100].

in the first 23 days, and of 7 given 600 r, 6 died in 18 days. But these authors claim that dogs given 1000 r survived 70 days (the total life-span for these dogs is not given, but it may be supposed greater). It is very improbable that the acute radiation sickness produced in dogs by 1000 r could last so long.

We consider that the disagreement with the published dose data is due to errors in the irradiation methods. Sufficiently general irradiation was not used. The focal distance is stated to vary with the size of the animal but the precise figures are not given. We can scarcely consider it permissible to compare results obtained with different irradiation conditions, because altering the focal distance changes the dose-rate. This was also evidently neglected.

This lack of attention to detail in the experiments casts doubt on the results. The data might mislead many readers and cannot be taken as reliable by specialists in the field.

We consider it essential to state the precise irradiation conditions in such work, as only if these are known can the results and conclusions be evaluated.

As it is important that irradiation conditions used with experimental animals should be standardized, and errors in this basic method are impermissible, we submit our letter for discussion.

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Translated by J. E. S. BRADLEY

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